"PHARMACOGNOSTICAL, PRELIMINARY PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITY OF EUPHORBIACEAE FAMILY CONTAINING PLANTS"

DISSERTATION

SUBMITTED TO

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In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

In

Department of Pharmacognosy

Under the Guidance and Supervision of

Mrs. T. KARTHIYAYINI, M. Pharm., (Ph.D)

Assoc. Professor

SUBMITTED BY

Reg No: 261420551

PADMAVATHI COLLEGE OF PHARMACY & RESEARCH INSTITUTE

PERIYANAHALLI-635205

DHARMAPURI, TAMIL NADU

APRIL – 2016

Dedicated to
The Almighty God,
My
Beloved Parents,
Teachers
And
Friends

CERTIFICATE

This is to certify that this dissertation entitled **"Pharmacognostical, Preliminary Phytochemical, And Pharmacological Activity Of Euphorbiaceae Family Containing Plants "** constitutes the original work carried out by **Mr.Sreehari.T.K B.Pharm,** under the guidance and supervision of **Mrs.T.Karthiyayini, M.Pharm., (Ph.D)** at the Department of Pharmacognosy , Padmavathi College of Pharmacy Research Institute, Periyanahlli, Dharmapuri – 635205

Prof.T.Karthyiyayini, M. Pharm., (Ph.D)
Department of Pharmacognosy
Padmavathi College of Pharmacy &
Research Institute,
Periyanahalli, Dharmapuri, Tamilnadu-635205.

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Head,
Dept. of Pharmacognosy,
Padmavathi College of Pharmacy &
Research Institute,
Periyanahalli, Dharmapuri, Tamilnadu-635205.

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PRINCIPAL,

Dr. K.L.SENTHIL KUMAR, M. Pharm., Ph.D.,

Padmavathi College of Pharmacy & Research Institute,

Periyanahalli, Dharmapuri, Tamilnadu-635205.

Place :

Date :

EVALUATION CERTIFICATE

This is to Certify that the bonafide dissertation work on **Pharmacognostical, Preliminary Phytochemical, and Pharmacological Activity of Euphorbiaceae Family Containing Plants"** submitted by **SREEHARI.T.K (261420551)** submitted to the Tamil Nadu Dr.M.G.R Medical University, Chennai, for the partial fulfillment of the requirement for the award of degree of Master of Pharmacy was evaluated.

Examination centre: Padmavathi College of Pharmacy & Research Institute, Periyanahalli, Dharmapuri, Tamilnadu-635205

Date:

1).Internal Examiner **2).External Examiner**

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Sreehari.T.K

(Reg No: 261420551)

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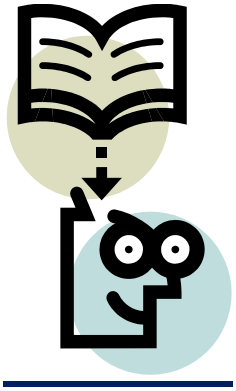
CHAPTER -I



Introduction

INTRODUCTION

India is said to be the paradise of herbs in the world. India has every possible agro-climatic zone from the highest of Himalayas the highest in the world down to the sea level. From the dry arid desert of Rajasthan to the wettest place in the world in Cheerapunjee.

From the oldest in the world part of Gondwana land in the south to the rich alluvium plains in the north. Thus all variety of herbs spices and aromatic plant can find suitable soil environmental conditions.

The potential use of herbal product is very wide Colouring of food, tanning of leather, plant protection, to make poison arrows, to produce gums and resins, or as a medicine. Of all the uses of herbs, the most important since time began has been their medicinal properties. Herb gardens were planted at the major universities in the 16^{th} and 17^{th} centuries to research the medicinal properties of herbs. Herbal dyestuff is also a vital source for preparing the natural colour. The leather, wool and cloth from which shoes and clothing were made were colored with dyestuff.

Many herbs give off scent, most of which we find pleasant or delightful, the aromatic properties of herbs must have been the first to be discovered by humans. Springer or leaves were picked to be taken home to spread on the floor where, when walk upon, wonderful fragrance were released in the home.

Pictures and name of herbs often adorn the packaging of cosmetic. These soften the skin, prevent dandruff, or have some other benefit for the skin or hair. Herbs which are used as soaps, skin lotion, shampoo, bath oil.

Some plants are poisonous and are used to kill insects or are smeared on the tip of arrow to kill warm blooded creatures. Other is used by people as narcotics to drug them, in order to forget their troubles or to cause hallucination. Sometime the herb is just used for its enjoyment, such as tobacco.

Herbs contain substances which can heal us or make us ill. This depends on the complaint, the dose taken, and the sensitivity for the substance which differs from person to person. Many people imagine that natural substances are less harmful than chemical ones but this is misguided. Some plants contain such powerful toxin that ingesting a small amount can be lethal.

Medicinal plants are used to treat illness and diseases for thousands of years. They have gained economical importance because of their application in pharmaceutical, cosmetic, perfumery and food industries. The interest in herbal systems of medicine is growing day-by-day because nature can cure many diseases (**Rekha Rajendran *et al.,* 2010**).

Medicinal plants are used in treatment of various diseases. *Asparagus racemosus, Withania somnifera, Glycerrhiza glabra* etc., are used in treatment of anaemia, *Piper longum, Adathoda vasica, Zingiber officinalis* etc., are used in bronchial asthma, *Terminalia chebula, Phyllanthus emblica, Ricinus communis* etc., and are used in Arthritis. *Terminalia chebula, phyllanthus emblica, Tribulus terestris* etc., are used in obesity. *Withania somnifera, Tribulus terestris, Zingiber officinalis* etc., are used in treatment of paralysis, *Piper longum, Zingiber officinale, cucuma longa, Ocimum sanctum* etc., are used to improve blood circulation, *Azadirachta indica, Holarrhena antidyseterica, Tinospora cordifolia* etc., and are used in cancer therapy **(Ramar Perumal Samy *et al.,* 2008)**

Medicinal plants of commercial significance include poppy, Isabgol, Senna, Cinchona, Ipecac, Belladonna, Ergot, Amla, Chirata, Kalmegh, Safed musli, Ashoka, Ashwagandha, Bael, Shatavari, Tulsi, Brahmi, Chandan, Pippali etc. Hotspots are areas of exceptional concentration of endemic species. Endemic species (Genera, Families) are restricted in their geographical distribution and do not occur outside these areas. Nearly 25 hotspots have been recognised worldwide (**Myers *et al.,* 2000**) which harbour 44% of all endemic plant species.

Among these areas, Western Ghats along Srilanka, Eastern Himalayas and Andaman-Nicobar Islands along with Indo-Burma regions are recognized as hotspots of India. Out of the

49,219 plant species, 5150 are endemic to India and distributed into 141 genera under 47 families corresponding to about 30% of the world's recorded flora, which means 30% of the world's recorded flora is endemic to India. Of these endemic species, 3,500 are found in the Himalayas and adjoining regions and 1600 in the Western Ghats alone.

If endemic plants are not protected, they may become extinct. The Govt of India has recognized some plant species which need to be conserved, they include: *Azadirachta indicia, Aegle marmelos, Andrographis paniculata, Asaparagus racemosus, Bauhinia vahlii, Emblica officinalis, Holorrhena antidysenterica, Gymnema sylvestre, Litsea glutinosa, Mallotus philippenensis, Pterocarpus marsupium, Soymida febrifuga, Strychnos potatorum, Sapindus emarginatus, Strychnos nux-vomica, Terminalia bellerica, Terminalia chebula* **(Ved and Goraya, 2007).** The Government of India has mounted a programme of Vanaspathi Van Project to promote Indian System of Medicine and for development of medicinal plants in degraded forests.

Diabetes is one of the major culprits responsible in degrading the health of a person in this stressful life. During world war-II when insulin was not available in many countries, search was made for a substitute for insulin from plant sources. Moreover drugs used in Type-2 have a number of limitations as they produce severe adverse effects and high rate of secondary failure (**Xie *et al.*, 2002).** Many plant species in folk medicine were used for their hypoglycemic properties and therefore used to treat diabetes **(Cragg *et al.*, 1997).** Some of the plants with anti diabetic activity include *Allium cepa, Coccinia indica, Ficus glomerata, Gymnema sylvestre, Momordica charantia, Pterocapus marsupium, Rauwolfia serpentina, Syzyygium cumini* (Kong, 2003). Plants with proven hypoglycemic effects were found to contain compounds like terpenoids **(Manikako *et al.*, 1997)** glycosides **(Das *et al.*, 1996) (Grove *et al.*, 2002),** Alkaloids **(Ivoora *et al.*, 1989)** and saponins **(Routhu *et al.*, 2005)** etc.

Liver is major functional organ in the body and its diseases are serious health problems which are encountered very commonly in present era. The cause for these problems may be drugs, chemicals, alcohol, environmental pollution etc. Conventional medical therapy for many common liver disorders, including non alcoholic fatty liver disease and viral hepatitis has limited efficacy and potentially life threatening side effects. Various medicinal plants are used in

traditional medicine for their hepato protective effects. The most commonly used medicinal plants for management of liver diseases include *Phyllanthus spp (Euphorbiacae) Silybum marianum, Glycerrhiza glabra* **(Kong, 2003)** etc.

Plants are considered to be biosynthetic innovatives, which produce primary and secondary metabolites. Many primary metabolites like carbohydrates, proteins and lipids and secondary metabolites like glycosides, alkaloids, tannins, volatile oils etc., which have therapeutic effects in human beings and animals are obtained from these solar powered biosynthetic laboratories. Secondary metabolites have been shown to alter biological processes which may reduce the risk of chronic diseases in humans. An impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicines **(Saini *et al.*, 2009).** Modern research has made it possible to isolate and identify active constituents from the extracts and to verify their therapeutic activity and specify dose-response relationship. Inspite of developments in synthetic chemistry, higher plants are still a source of the medicinal compounds. With a view to explore traditional medicines and to investigate their scientific application, an endemic medicinal plant ***Phyllanthus emblica* Linn.,** and ***Phyllanthus amarus***, which has been used as a traditional folklore medicine, was selected for the present work.

***Phyllanthus emblica* Linn**. [Syn: *Emblica officinalis* Gaertn.] (Euphorbiaceae) is popularly known as '*Amla'* or '*Awala'* in Maharashtra. It is a tree of small or moderate size with a greenish-grey bark and greenish yellow flowers, formed in axillary clusters. The feathery leaves are linear-oblong, with a rounded base and obtuse or acute apex. The tender fruits are green, fleshy, globose and shining, and change to light yellow or brick-red when mature. It grows in tropical and subtropical parts of China, India, Indonesia, and on the Malay Peninsula. The Malaysian variety has more scurfy branchlets and the immature fruit is top-shaped.

The fruits are known as Amalakam and Sriphalam in Sanskrit, Emblic myrobalam and Indian gooseberry in English, and Phylontha emblic in French. It is a well known remedy for the treatment of various types of disorders in the ayurvedic and homoeopathic systems of medicine in India. Its fruit contains a series of diterpenes referred to as the gibberellins, as well as the triterpene lupeol, flavonoids and polyphenols. It also shows the presence of phyllantine and

zeatin alkaloid and a number of benzenoids including amalic acid, corilagin,ellagic acid, 3-6-diO-galloyl-glucose, ethyl gallate, 1,6-di-O-galloyl-ß-Dglucose, 1-di Ogalloyl- ß-Dglucose, putranjivain A, digallic acid, emblicol and alactaric acid. This fruit also contains significantly high amounts of ascorbic acid (vitamin C)[25]

***P. emblica* L**. has been used for anti-inflammatory and antipyretic treatments by rural populations in its growing areas. Malays use a decoction of its leaves to treat fever[6]. In Indonesia, the pulp of the fruit is smeared on the head to dispel headache and dizziness caused by excessive heat[7]. The earlier chemical findings and biological activities have since been confirmed with more advanced techniques. Active principles or extracts of ***P. emblica* L**. have been shown to possess several pharmacological actions, *e.g.* analgesic, anti-inflammatory, antioxidant, chemoprotective, hypolipidaemic and anti- HIV-1 (Human immunodeficiency virus-1) activities[2,8-14].

The fruit is rich in source of pectin and contains gallic acid, ellagic acid and glucose in its molecule which is naturally present in the fruit, prevents or retards the oxidation of vitamin-C and renders the fruit a valuable antiscorbutic, in the fresh as well as in the dry condition[15]. Tannin is found in different organs as fruit, twig bark, stem bark and leaf. In view of its varied medicinal importance and to ensure the quality of the drug, the present pharmacognostic investigation and antioxidant studies on the fruits of ***Phyllanthus emblica*** and ***Phyllanthus amarus*** have been undertaken.

INTRODUTION ABOUT THE PLANT

PHYLLANTHUS EMBLICA (AMLA)

The world craves new ideas and looks to the Far East and Asia for inspiration and innovation. One Indian plant stands out as being exceptional for its ethnic, ethnobotanical and ethnopharmaceutical use. ***Phyllanthus emblica,*** also known as Emblica officinalis, or Amla, is one of the most frequently used of the Ayurvedic herbs. The plant, a member of the family Euphorbiaceae, grows to become a medium-sized tree. The fruit is similar in appearance to the common gooseberry, which is botanically unrelated to Amla. However, because of these similarities, Amla is often called **"Indian gooseberry."**

Amla is also valued as a natural antioxidant and a rich natural source of Vitamin C. Amla fruit is acrid, cooling, refrigerant, diuretic and laxative. Although just a dimunitive one inch in diameter, one Amla fruit has the same ascorbic acid content as two oranges. Because of this, Amla fruit juice, sediment and residue, have powerful antioxidant properties. The use of amla as an antioxidant has been examined by a number of authors [Bhattacharya; Chaudhuri] [28].

Experiments conducted at the Niwa Institute of Immunology in Japan have shown Amla to be a potent scavenger of free radicals. The studies showed that Amla preparations contained high levels of the free-radical scavenger, superoxide dimutase (SOD), in the experimental subjects.

Amalaki or Indian goose berry is also known as King of all medicinal plants. It is most important drug in Indian traditional system, especially Ayurveda. It has occupied major place in Ayurvedic medicines. It is a small medium size tree. The leaves are feathery with small oblong pinnately arranged leaflets. The tree is characteristic greenish grey with smooth bark. Amalaki possesses the highest level of heat and storage stable vitamin C known to man. The study includes macroscopy, microscopy, preliminary phytochemical screening and physic-chemical evaluation.

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Fig. No: I. 1 Exomorphic feature of ***Phyllanthus emblica*** and its twig

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Fig. No: I. 2 Fruits of ***Phyllanthus emblica***

Fig. No: I.3 Exomorphic feature of *Phyllanthus emblica*

Fig. No: I. 5 L.S. of *Phyllanthus emblica*

***PHYLLANTHUS AMARUS* (NIRURI)**

Phyllanthus amarus originated in India, usually occurring as a winter weed throughout the hotter parts. *The* ***Phyllanthus*** genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas. ***Phyllanthus amarus*** is an herb of Euphorbiaceae family that grows upto 60 cm. ***Phyllanthus*** means "**leaf** and **flower"** because the flowers, as well as the fruit, seem to become one with the leaf.

Phyllanthus amarus L., (Syn. P. fraternus Webster), Euphorbiaceae, is a common kharif (rainy season) weed found in both cultivated fields and wastelands. Recently it has attracted the attention of researchers, because of its hepatoprotective properties. No effective specific therapy is available for viral hepatitis but ***P. amarus*** has shown clinical efficacy in viral Hepatitis B .It is known for its liver healing properties so used in Chinese medicine for treatment of liver disease.

FigI.6 Exomorphic figure of ***Phyllanthus Amarus*** *and its twig*

FigI.7 Exomorphic figure of ***Phyllanthus Amarus*** *and its twig*

CHAPTER - II

Literature Review

LITERATURE REVIEW

I *PHYLLANTHUS EMBLICA*

A) PHARMACOLOGICAL STUDY

1) **Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK**. Antioxidant activity of active tannoid principles of Emblica officinalis (amla). **Ind J Exp Biol** 1999; 37: 676-80.

Abstract:

The antioxidant activity of tannoid active principles of ***E. officinalis*** consisting of emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%), was investigated on the basis of their effects on rat brain frontal cortical and striatal concentrations of the oxidative free radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), and lipid peroxidation, in terms of thiobarbituric acid-reactive products. The results were compared with effects induced by deprenyl, a selective monoamine oxidase (MAO) B inhibitor with well documented antioxidant activity. The active tannoids of E. officinalis (EOT), administered in the doses of 5 and 10 mg/kg, i.p., and deprenyl (2 mg/kg, i.p.), induced an increase in both frontal cortical and striatal SOD, CAT and GPX activity, with concomitant decrease in lipid peroxidation in these brain areas when administered once daily for 7 days. Acute single administration of EOT and deprenyl had insignificant effects. The results also indicate that the antioxidant activity of E. officinalis may reside in the tannoids of the fruits of the plant, which have vitamin C-like properties, rather than vitamin C itself.

2) **Vasudha Shukla , Manish Vashistha and Som Nath Singh** Evaluation of antioxidant profile and activity of amlaki (Emblica officinalis). Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi- 110054.

Abstract:

Aqueous and alcoholic extracts of amalki (Emblica officinalis), spirulina and wheatgrass were prepared and analyzed for antioxidant vitamin content (vitamin C and E), total phenolic

compounds. Antioxidant status, reducing power and effect on glutathione S-transferase (GST) activity were evaluated in vitro. Vitamin C content of crude amalaki powder was found to be 5.38 mg/g, while very less amount 0.22 mg/g was detected in wheat grass. Amalki was rich in vitamin E like activity, total phenolic content, reducing power and antioxidant activity. Total antioxidant activity of aqueous extract of amalki, spirulina and wheat grass at 1mg /ml concentration were 7.78, 1.33 and 0.278 mmol/l respectively. At similar concentrations the total antioxidant activity of alcoholic extract of amalaki, spirulina and wheat grass was 6.67, 1.73 and 0.380 mmol/l respectively. Amalki was also found to be rich source of phenolic compounds (241mg/g gallic acid equivalent). Alcoholic extract of wheat grass showed 50 % inhibition in FeCl2 - ascorbic acid induced lipid peroxidation of rat liver homogenates in vitro. Both aqueous and alcoholic extracts of amalaki inhibited activity of rat liver glutathione S-transferase (GST) in vitro in dose dependant manner. Since GST acts as powerful drug metabolizing enzyme its inhibition by amalaki offers possibility of its use for lowering therapeutic dose of herbal preparations. The aqueous extracts of both amalki and spirulina also showed protection against t-BOOH induced cytotoxicity and production of ROS in cultured C6 glial cells.

3) **Gulati RK, Agarwal S,Agrawal SS** 1995 Hepatoprotective studies on ***Phyllanthus emblica Linn***, and quercetin. Indian Journal of Experimental Biology 33(4):261.

Abstract:

Phyllanthus emblica is a constituent of many hepatoprotective formulations available in market. 50% alcoholic extract of P. emblica and quercetin isolated from it were studied for hepatoprotective effect against country made liquor (CML) and paracetamol challenge in albino rats and mice respectively. The extract at the dose of 100 mg/100 g [corrected], po and quercetin at the dose of 15 mg/100 g, po, produced significant hepatoprotection.

4) **Ihantola-Vormisto A, Summanen J, Kankaanranta H, Vuorela H, Asmawi ZM, Moilanen E** 1997 Anti-inflammatory activity of extracts from leaves of ***Phyllanthus emblica***. **Planta Medical** 63(6):518.

Abstract:

Leaves and fruits of ***Phyllanthus emblica L***. have been used for the anti-inflammatory and antipyretic treatment of rural populations in its growing areas in subtropical and tropical parts of China, India, Indonesia, and the Malay Peninsula. In the present study, leaves of ***P. emblica*** were extracted with ten different solvents (n-hexane, diethyl ether, methanol, tetrahydrofuran, acetic acid, dichloromethane, 1,4-dioxane, toluene, chloroform, and water). The inhibitory activity of the extracts against human polymorphonuclear leukocyte (PMN) and platelet functions was studied. Methanol, tetrahydrofuran, and 1,4-dioxane extracts (50 micrograms/ml) inhibited leukotriene B4-induced migration of human PMNs by 90% and N-formyl- L-methionyl-L-leucyl-L-phenylalanine (FMLP)-induced degranulation by 25-35%. The inhibitory activity on receptor-mediated migration and degranulation of human PMNs was associated with a high proportion of polar compounds in the extracts as assessed by normal phase thin layer chromatography. Diethyl ether extract (50 micrograms/ml) inhibited calcium ionophore A23187-induced leukotriene B4 release from human PMNs by 40%, thromboxane B2 production in platelets during blood clotting by 40% and adrenaline-induced platelet aggregation by 36%. Ellagic acid, gallic acid and rutin, all compounds isolated earlier from ***P. emblica***, could not explain these inhibitory activities on PMNs or platelets by ***P. Emblica***

5) **Mathur R, Sharma A, Dixit VP, Varma M 1 996** Hypolipidaemic effect of fruit juice of Emblica offitinalis in cholesterol-fed rabbits. **Journal of Ethnopharmacology** 50:61

Abstract:

The lipid lowering and antiatherosclerotic effects of Emblica officinalis (Amla) fresh juice was evaluated in cholesterol-fed rabbits (rendered hyperlipidaemic by atherogenic diet and cholesterol feeding). E. officinalis fresh juice was administered at a dose of 5 ml/kg body weight per rabbit per day for 60 days. Serum cholesterol, TG, phospholipid and LDL levels were lowered by 82%, 66%, 77% and 90%, respectively. Similarly, the tissue lipid levels showed a significant reduction following E. officinalis juice administration. Aortic plaques were regressed. E. officinalis juice treated rabbits excreted more cholesterol and phospholipids, suggesting that the

mode of absorption was affected. ***E. officinalis*** juice is an effective hypolipidaemic agent and can be used as a pharmaceutical tool in hyperlipidaemic subjects.

B) PHARMACOGNOSTICAL STUDY

6) **Bharambe Swati Vasant*1, Darekar Avinash Bhaskarrao1, Saudagar Ravindra Bhanudas2** Emblica Officinalis – The Wonder Of Ayurvedic Medicine. **World Journal Of Pharmacy And Pharmaceutical Sciences** Volume 3, Issue 1, 285-306. Review Article Issn 2278 – 4357

Abstract:

Medicinal plants are natural gift to human lives to promote disease free healthy life. ***Emblica officinalis***, commonly known as amla is widely distributed in tropical and subtropical areas and has therapeutic potential against deleterious diseases. Earlier it becomes a notable fruit for its rich amount of vitamin C, polyphenols such as tannins, gallic acid, ellagic acid, flavonoids like quercetin and rutin. ***Emblica officinalis*** (Amla) are widely used in the Indian system of medicine and believed to increase defense against diseases. This article discuses and summarizes important medicinal values of Emblica officinalis (EO). In this communication, we reviewed the applications of EO in hepatoprotective, antioxidant, anti-inflammatory, dental problem, respiratory problem and various other diseases. These papers also review the studies on the Amla as important medicinal values.In this communication, we reviewed the applications of EO in cancer, diabetis, liver treatment, heart disease, ulcer, anemia and various other diseases. The use of EO as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastro protective are also reviewed. Its applications for memory enhancing, ophthalmic disorders, lowering cholesterol level are focused. The effects of EO in neutralizing snake venom and as an antimicrobial are also included. This paper also review the retrospective studies on the Amla at molecular level.

7) **Khorana, M.L; Rao, M.R.R; Siddiqui, H.H** "Antibacterial and antifungal activity of ***Phyllanthus emblica Linn***." **Indian. J. Pharm.,** 1959; 21: 331.

Abstract:

Sanjivani Vati is official in Ayurvedic formulary of India and is prescribed for the treatment of cough and fever. It is a polyherbal preparation containing ten ingredients. Sanjivani Vati is a diaphoretic formula that increases sweating. It is a detoxifying formula that reduces aama. This "Aama" leads to blockage in channels and many diseases like high cholesterol, blocked coronary arteries, piles, auto-immune disorders like sarcoidosis, rheumatoid arthritis. The ingredients of Sanjivani Vati are universal digestive aid, antihelminthic, diuretic, carminative etc. This review explains the potential of Sanjivani Vati which helps the researcher to explode more about this ayurvedic formulation

8) **Saeed S, Tariq P.** Antibacterial activities of ***Emblica officinalis*** and Coriandrum sativum against Gram negative urinary pathogens. **Pak J Pharm Sci.** 2007 Jan; 20(1):32-5.

Abstract:

Present investigation is focused on antibacterial potential of aqueous infusions and aqueous decoctions of ***Emblica officinalis*** (amla) and *Coriandrum sativum* (coriander) against 345 bacterial isolates belonging to 6 different genera of Gram negative bacterial population isolated from urine specimens by employing well diffusion technique. Aqueous infusion and decoction of ***Emblica officinali***s exhibited potent antibacterial activity against Escherichia coli (270), Klebsiella pneumonia (51), K. ozaenae (3), Proteus mirabilis (5), Pseudomonas aeruginosa (10), Salmonella typhi (1), S. paratyphi A (2), S. paratyphi B (1) and Serratia marcescens (2) but did not show any antibacterial activity against Gram negative urinary pathogens

C) PHYTO-CHEMIICAL STUDY

9) **Habib-ur-Rehman, Yasin KA, Choudhary MA, *et al.*** (Jul 2007). "Studies on the chemical constituents of ***Phyllanthus emblica***". **Nat. Prod. Res.** 21 (9): 775–81. Doi:10.1080/14786410601124664. PMID 17763100.

Abstract:

Phytochemical investigations on ***Phyllanthus emblica*** have resulted in the isolation of the two new flavonoids, kaempferol-3-O-alpha-L-(6"-methyl)-rhamnopyranoside (1) and kaempferol-3-O-alpha-L-(6"-ethyl)-rhamnopyranoside (2). Their structures were determined on the basis of extensive spectroscopic studies including 2D-NMR experiments. determined on the basis of extensive spectroscopic studies including 2D-NMR experiments.

10) **Naik G.H., Priyadarsini K.I., Bhagirathi R.G., Mishra B., Mishra K.P., Banavalikar M.M., Mohan H.** In-vitro antioxidant studies and free radical reactions of *triphala*, an ayurvedic formulation and its constituents. ***Phytother. Res.*** 2005; **19:582**–586.

Abstract:

The aqueous extract of the fruits of ***Emblica officinalis (***T1), *Terminalia chebula* (T2) and *Terminalia belerica* (T3) and their equiproportional mixture triphala were evaluated for their in vitro antioxidant activity. gamma-Radiation induced strand break formation in plasmid DNA (pBR322) was effectively inhibited by triphala and its constituents in the concentration range 25-200 microg/mL with a percentage inhibition of T1 (30%-83%), T2 (21%-71%), T3 (8%-58%) and triphala (17%-63%). They also inhibited radiation induced lipid peroxidation in rat liver microsomes effectively with IC (50) values less than 15 microg/mL. The extracts were found to possess the ability to scavenge free radicals such as DPPH and superoxide. As the phenolic compounds present in these extracts are mostly responsible for their radical scavenging activity, the total phenolic contents present in these extracts were determined and expressed in terms of gallic acid equivalents and were found to vary from 33% to 44%. These studies revealed that all three constituents of triphala are active and they exhibit slightly different activities under different conditions. T1 shows greater efficiency in lipid peroxidation and plasmid

DNA assay, while T2 has greater radical scavenging activity. Thus their mixture, triphala, is expected to be more efficient due to the combined activity of the individual components.

11) **Yakhmi JV, Banavaliker MM, Biyani MK, Mittal JP**. Characterizing the antioxidant activity of amla (Phyllanthus emblica) extract. **Curr Sci** 2001; 81:185-90.

Abstract:

Aques and alcoholic extract of amlaki (Emblica officinalis) spirulina andwheatgrass were prepared and analyzed for antioxidant vitamin content (vitamin C and E), total phenolic compounds. Antioxidant status, reducing power and effect on glutathione S-transferase (GST) activity were evaluated in vitro. Vitamin C content of crude amalaki powder was found to be 5.38 mg/g, while very less amount 0.22 mg/g was detected in wheat grass. Amalki was rich in vitamin E like activity, total phenolic content, reducing power and antioxidant activity. Total antioxidant activity of aqueous extract of amalki, spirulina and wheat grass at 1mg/ml concentration were 7.78, 1.33 and 0.278 mmol/l respectively. At similar concentrations the total antioxidant activity of alcoholic extract of amalaki, spirulina and wheat grass was 6.67, 1.73 and 0.380 mmol/l respectively. Amalki was also found to be rich source of phenolic compounds (241mg/g gallic acid equivalent). Alcoholic extract of wheat grass showed 50 % inhibition in $FeCl_2$-ascorbic acid induced lipid peroxidation of rat liver homogenates in vitro. Both aqueous and alcoholic extracts of amalaki inhibited activity of rat liver glutathione S-transferase (GST) in vitro in dose dependant manner. Since GST acts as powerful drug metabolizing enzyme its inhibition by amalaki offers possibility of its use for lowering therapeutic dose of herbal preparations. The aqueous extracts of both amalki and spirulina also showed protection against t-BOOH induced cytotoxicity and Production of ROS in cultured C_6 glial cells

II *PHYLLANTHUS AMARUS*

A) PHARMACOLOGICAL ACTION

Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J (December 2006). Phytochemicals from ***Phyllanthus amarus Linn***. and their pharmacological properties: a review". **The Journal of Pharmacy and Pharmacology** 58 (12): 1559–70.

Abstract:

This review discusses the medicinal plant ***Phyllanthus amarus Linn.*** (Euphorbiaceae), its wide variety of phytochemicals and their pharmacological properties. The active phytochemicals, flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts of P. amarus. Extracts of this herb have been proven to have therapeutic effects in many clinical studies. Some of the most intriguing therapeutic properties include anti-hepatotoxic, anti-lithic, anti-hypertensive, anti-HIV and anti-hepatitis B. Therefore, studies relating to chemical characteristics and structural properties of the bioactive phytochemicals found in P. amarus are very useful for further research on this plant as many of the phytochemicals have shown preclinical therapeutic efficacies for a wide range of human diseases, including HIV/AIDS and hepatitis B.

12) **Faremi, T., *et al*.** "Hepatoprotective potentials of ***Phyllanthus amarus*** against ethanol-induced oxidative stress in rats**." Food Chem. Toxicol**. 2008; 46(8): 2658-6.

Abstract:

The hepatoprotective effect of methanolic extract of the leaf of ***Phyllanthus amarus*** (P. amarus) against ethanol-induced oxidative damage was investigated in adult male Wistar albino rats. P. amarus (250 and 500 mg/kg/day) and ethanol (5 g/kg/day, 20% w/v) were administered orally to animals for 4 weeks and 3 weeks, respectively. Ethanol treatment markedly decreased the level of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in the liver, which were significantly enhanced by P. amarus treatment. Glutathione-S transferase (GST), which was increased after chronic ethanol administration, was significantly reduced by ***P. amarus*** treatment in the liver. Also, ***P. amarus*** significantly increased the activities of

hepatic alanine transaminase (ALT) and aspartate transaminase (AST) as well as alkaline phosphatase (ALP), with a concomitant marked reduction in the plasma activity of the transaminases in the ethanol-challenged rats. Lipid peroxidation level, which was increased after chronic ethanol administration, was significantly reduced in the liver by ***P. amarus*** co-treatment. Results show that P. amarus leaf extract could protect the liver against ethanol-induced oxidative damage by possibly reducing the rate of lipid peroxidation and increasing the antioxidant defence mechanism in rats.

B) PHARMACOGNOSTICAL STUDY

13) **Khatoon,S.,*et al.*** "Comparative pharmacognostic studies of three ***Phyllanthus*** species." 2006 Mar; 104(1-2): 79-86.

Abstract:

Different species of ***Phyllanthus*** are considered to be very effective hepatoprotective agents in the Indian indigenous systems of medicine and are considered bitter, astringent, stomachic, diuretic, febrifuge, deobstruant and antiseptic. Still ayurvedic practitioners prescribed fresh juice of 'Bhuiamlki' for jaundice. Various species of ***Phyllanthus*** are being sold in India under the trade name 'Bhuiamlki'. During market surveillance of herbal drug, it was observed that almost all the commercial samples, either comprise of Phyllanthus amarus Schum & Thonn. or *Phyllanthus maderaspatensis Linn*. or mixture of *Phyllanthus amarus, Phyllanthus fraternus Webste*r. and *Phyllanthus maderaspatensis*. Therefore, in this context the detailed pharmacognostical evaluation of all the three species has been carried out with the aim to establish the identification markers of this important hepatoprotective agent (effective in hepatitis B too). The study conclude that all the three species can be differentiated on the basis of macro and microscopic characters, physico-chemical values, HPTLC fingerprint profile, and detection of phyllanthin and hypophyllanthin as marker components. Besides, an interesting conclusion can also be drawn that phyllanthin and hypophyllanthin said to protect hepatocytes against carbon tetrachloride and galactosamine induced toxicity, may not be exclusively responsible for hepatoprotective activity as these are present only in ***Phyllanthus amarus*** while *Phyllanthus fraternus* and *Phyllanthus maderaspatensis* also possess significant hepatoprotective activity.

14) **Naik AD, Juvekar AR** (September 2003). "Effects of alkaloidal extract of ***Phyllanthus amarus*** on HIV replication". **Indian Journal of Medical Sciences** 57 (9): 387-93

Abstract:

Phyllanthus amarus has been found to exhibit marked inhibitory effect on hepatitis B virus evident by its exhaustive utility in cases of chronic jaundice. However, till date, research has not been focused on identification and validation of active pharmacophores of ***Phyllanthus amarus*** responsible for the reported inhibitory effect of its aqueous extract on anti-human immunodeficiency virus. The present investigation examines the anti-HIV effects of the alkaloidal extract of ***Phyllanthus amarus*** in human cell lines. The inhibitory effect on HIV replication was monitored in terms of inhibition of virus induced cytopathogenecity in MT-4 cells. The alkaloidal extract of Phyllanthus amarus showed suppressing activity on strains of HIV-1 cells cultured on MT-4 cell lines. The CC50 for the extract was found to be 279.85 microgmL (-1) whereas the EC50 was found to be 20.98 microgmL (-1). Interestingly the Selectivity Index (SI) was found to be 13.34, which showed a clear selective toxicity of the extract for the viral cells. The alkaloidal extract of ***Phyllanthus amarus*** was thus found to exhibit sensitive inhibitory response on cytopathic effects induced by both the strains of human immunodeficiency virus on human MT-4 cells in the tested concentrations.

C) PHYTO-CHEMICAL STUDY

15) **T.P.A. Devasagayam, J.C.Tilak, K.K.Boloor, K.S.Sane and R.D.Lele,** Review: Free radicals and antioxidants in human health: current status and future prospects, **J. Assoc. phys. India** 52(2004)794-804.

Abstract:

Free radicals and related species have attracted a great deal of attention in recent years. They are mainly derived from oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS), and are generated in our body by various endogenous systems, exposure to different physicochemical conditions or pathophysiological states. Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations. Free radical damage to

protein can result in loss of enzyme activity. Damage caused to DNA, can result in mutagenesis and carcinogenesis. Redox signaling is a major area of free radical research that is attracting attention. Nature has endowed us with protective antioxidant mechanisms- superoxide dismutase (SOD), catalase, glutathione, glutathione peroxidases and reductase, vitamin E (tocopherols and tocotrienols), vitamin C etc., apart from many dietary components. There are epidemiological evidences correlating higher intake of components/ foods with antioxidant abilities to lower incidence of various human morbidities or mortalities. Current research reveals the different potential applications of antioxidant/free radical manipulations in prevention or control of disease. Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity. Newer and future approaches include gene therapy to produce more antioxidants in the body, genetically engineered plant products with higher level of antioxidants, synthetic antioxidant enzymes (SOD mimics), novel biomolecules and the use of functional foods enriched with antioxidants.

CHAPTER -III

Plan of work

PLAN OF WORK

In this present study we aim to study the antioxidant activity of different plants from Euphorbiaceae family. These three plants have more similar characters in their morphology and also have some similar chemical nature. So a comparative work for their antioxidant activity was planned for which the proceedings are as below.

1. Collection of the plant.
2. Plants Authentification.
   Plant Anatomy Research Centre
   W.Tambaram, Chennai-45
3. Collection of Literature
4. Plant material to be cleaned, dried, and powdered.
5. Preparation of Methanolic extracts by cold maceration method.
6. Pharmacognostical Evaluation.
7. Physico-chemical and Phytochemical study.
   A. Extractive value
   B. Ash value
   C. Fibre content
   D. Phytochemical test
   E. Chromatographic Evaluation
      i. TLC
      ii. HPTLC
   F. Vitamin C estimation
8. Anti oxidant study.
   a) Scavenging of hydrogen peroxide radicals.
   b) DPPH free radical scarving activity

CHAPTER - IV

pharmacognostical study

i) MACROSCOPY

BOTANICAL INFORMATION OF PLANT IDENTIFICATION[2,5,9,12,13,23,2,29,315]

A) *Phyllanthus emblica* [9, 12, 13]

Synonym - Amla, Gooseberry, Emblic Myrobalam

Botanical name - ***Phyllanthus emblica* Linn.**

Botanical classification:

Kingdom	–	Plantae - Plants
Division	–	Angiospermae
Class	–	Dicotyledonae
Order	–	Geraniales
Family	–	Euphorbiaceae
Genus	–	*Emblica*
Species	–	*officinalis* Geartn**.**

Vernacular name of plant :

Amalaki	-	Sanskrit
Amla, Amlaki	-	Bengali
Ambala, Anvala	-	Gujarati
Amla, Amlika	-	Hindi
Amalaka, Nelli	-	Kannada
Nelli	-	Malayalam
Avala	-	Marathi
Nellikai	-	Tamil
Usirikai	-	Telugu

Geographical Source :

Amalaki is native to tropical southeastern Asia, particularly in central and southern India, Pakistan, Bangladesh, Sri Lanka, Malaysia, southern China and the Mascarene Islands. It is commonly cultivated in gardens throughout India and grown commercially as a medicinal fruit.

Habitat:

Amla is native to the plains lower mountainous regions of the Indian subcontinent. It grows at elevations of 200 to nearly 2000 meters above sea level. Amla's natural habitat extends from Burma on the east to Afghanistan in the west, and from Deccan in south India to the foothills of the Himalayan range.

Cultivation and collection:

The tree is small to medium sized, reaching 8 to 18 m in height, with a crooked trunk and spreading branches. The branchlets are glabrous or finely pubescent, 10–20 cm long, usually deciduous; the leaves simple, subsessile and closely set along branchlets, light green, resembling pinnate leaves. The flowers are greenish-yellow. The fruit is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with 6 vertical stripes or furrows.

Ripening in autumn, the berries are harvested by hand after climbing to upper branches bearing the fruits. The taste of Indian gooseberry is sour, bitter and astringent, and is quite fibrous. In India, it is common to eat gooseberries steeped in salt water and turmeric to make the sour fruits palatable. It is used to straighten hair.

Description about Plant[9, 12, 13, 25]

Macroscopic description: Amla consists of curled pieces of pericarp of dried fruit occurring either as separated single segment; 1-2 cm long or united as 3 or 4 segments; bulk colour grey to black, pieces showing, a broad, highly shrivelled and wrinkled external convex surface to somewhat concave, transversely wrinkled lateral surface, external surface shows a few whitish specks, occasionally some pieces show a portion of stony testa (which should be removed before processing); texture rough, cartilaginous, tough; taste, sour and astringent.

A small to medium sized deciduous tree 8-18 meters height with thin light grey bark exfoliating in small thin irregular flakes. Fruit, globose, 2.5-3.5 cm in diameter, fleshy, smooth with six prominent lines; greenish when tender, changing to light yellowish or pinkish colour when mature, with a Few dark specks: taste, sour and astringent followed by delicately sweet taste.

Compound Leaf:

Leaves are simple, sub sessile, closely set along the branchlets, light green having the appearance of pinnate leaves.

Flower:

Flowers are greenish yellow, in auxillary fascicles, unisexual, males numerous on short slender pedicels, females few, sub sessile, ovary 3-celled.

Fruits:

Fruits globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in 2-seeded 3 crustaceous cocci1.

Uses: [2, 9, 13, 23]

The fruits are sour, astringent, bitter, acrid, sweet, cooling, anodyne, ophthalmic, carminative, digestive, stomachic, laxative, alterant, aphrodiasic, rejuvenative, diuretic, antipyretic and tonic. They are useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair.

B) *Phyllanthus amarus* [6,9,17]

Synonym :- ***Phyllanthus amarus***

Botanical name :- ***Phyllanthus niruri***

Botanical classification:

Kingdom	–	Plantae
Division	–	Magnoliophyta
Class	–	Magnoliopsida
Order	–	Malpighiales
Family	–	Euphorbiaceae
Genus	–	*Phyllanthus*
Species	–	*P. amarus*

Vernacular name of plant :

Bhumyamalaki	-	Sanskrit
Bhuimala	-	Bengali
Jamgli-amli	-	Hindi
Kirunelli	-	Kannada
Kilarnelli	-	Malayalam
Bhuivali	-	Marathi
Kilannelli	-	Tamil Nadu

Geographical Source :

India, Bangladesh, West Indies, Fiji, Peru, Virgin Island, Haiti, Sudan, Brazil, France, Mexico, Paraguay, Thailand, Srilanka.

Habitat :

Common in central and southern India extending to Srilanka.

Cultivation and collection :

Phyllanthus amarus originated in India, usually occurring as a winter weed throughout the hotter parts. The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas. ***Phyllanthus amarus*** is a herb of Euphorbiaceae family that grows up to 60 cm. *Phyllanthus* means "leaf and flower" because the flower, as well as the fruit, seem to become one with the leaf.

Phyllanthus amarus L., (Syn. *P. fraternus* Webster), Euphorbiaceae, is a common kharif (rainy season) weed found in both cultivated fields and wastelands. Recently it has attracted the attention of researchers, because of its hepatoprotective properties. No effective specific therapy is available for viral hepatitis but ***P. Amarus*** has shown clinical efficacy in viral Hepatitis B .It is known for its liver healing properties so used in Chinese medicine for treatment of liver diseases.

Description About Plant[6, 9, 17]

Phyllanthus amarus is an annual herb and its height varies between 30–60 cm.

Compound Leaf:

Stem is angular with numerous distichous, elliptic-oblong leaves.

Flower:

Flowers are yellow and very numerous, monoecious with 1–3 staminate flowers and solitary pistillate flower borne axillary.

Fruits:

Fruits capsule, very small, globose, smooth, seeds 3-gonous, longitudinally ribbed on the back. Seed to seed cycle occurs in two or four weeks.

Uses:

Its root, leaves, fruits, milky juice, and whole plants are used as medicine. According to Ayurvedic system of medicine it is considered acrid, cooling, alexipharmic and useful in thirst, bronchitis, leprosy, anemia, urinary discharge, anuria, biliousness, asthma, for hiccups, and as a

diuretic. According to Unani system of medicine herb is stomachic and good for sores and useful in chronic dysentery. Fruits useful for tubercular ulcers, wounds, sores, scabies and ring worm.

ii) MICROSCOPY

Introduction

Microscopy is an important tool in the evaluation of crude drugs which is applicable at various levels such as the Authentification of crude drugs, study of powdered drugs, calcium oxalate crystals, starch grains, pollen grains etc. Ash values, extractive values and foaming index are used for the study of physical properties.

Arrangement of plant into groups and subgroups is commonly spoken as classification. Various systems of classifying plants have gradually developed during past few centuries, which have emerged as a discipline of botanical science known as taxonomy or systemic botany. The word **'taxonomy'** is derived from two Greek words **'Taxis'** meaning as arrangement and 'Nomas' meaning laws. Therefore, the systemization of our knowledge about plants in an orderly manner becomes subject matter of systematic botany**.**

The aim and objective of taxonomy is to discover the similarities and differences in the plants, including their closed relationship with their descents from common ancestry. It is a scientific way of naming, describing and arranging the plant in an orderly manner.

Materials and Methods for Anatomical Studies

A. Preparation of specimen

Most care was taken to select healthy plant and for normal organs. The required sample of Different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid -5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1944. Infiltration of the specimens was carried by gradual addition of paraffin wax (m. pt. 58-60 ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

B. Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtone. The thickness of the section was 10-12 µm. Dew axing of the sections was by customary procedure

(Johansen, 1940)[31]. The sections were stained with Toluidine blue as per the method published by **O'Brien et al. (1964)[35].** Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections were also stained with saffranin and Fast-green and IKI (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid **(Sass, 1940)[30]** were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component were studied and measured.

C. Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with **Nikon Lab photo 2** Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books **(Esau, 1964).[28]**

A) ***Phyllanthus emblica***

Amalaki (fresh fruit pulp)

Amalaki consists of fresh fruit pulp of ***Emblica officinalis*** **Gaertn**. (Fam. Euphorbiaceae), Syn. ***Phyllanthus emblica*** **Linn.** mostly collected in winter season after ripening and in Kashmir in summer, a small or medium sized tree, found in mixed deciduous forests, ascending to 1300 m on hills and cultivated in gardens and home yards.

Microscopic characteristics:

Transverse section of mature fruit shows an epicarp consisting of single layer of epidermis and 2-4 layers of hypodermis; epidermal cell, tabular in shape, covered externally with a thick cuticle and appears in surface view as polygonal; hypodermal cells tangentially elongated, thick-walled, smaller in dimension than epidermal cells.

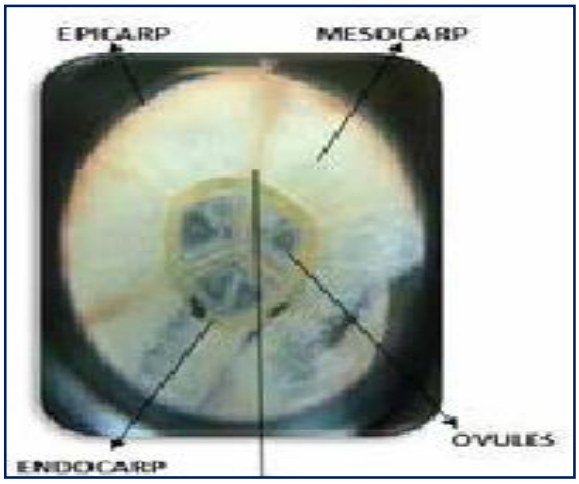


Fig IV. 1 T.S. of Mesocarp of Amalaki

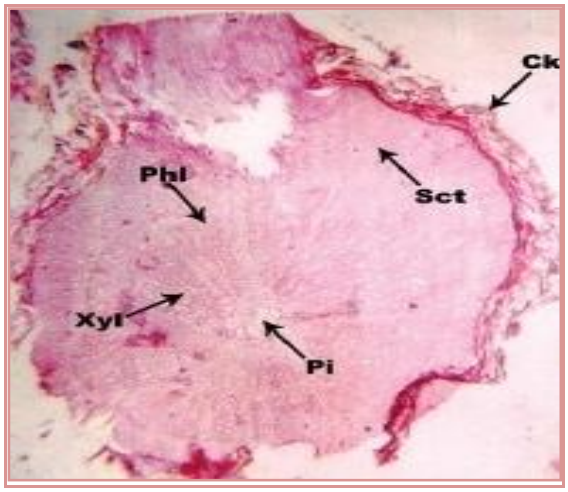


Fig IV. 2 T.S. of Amalaki (upper view)

Mesocarp forms bulk of fruit, consisting of thin-walled parenchymatous cells with intercellular spaces, peripheral 6-9 layers smaller, ovoid or tangentially elongated while rest of cells larger in size, isodiametric and radially elongated; several collateral fibrovascular bundles scattered throughout mesocarp consisting of xylem and phloem; xylem composed of tracheal elements,

fibre tracheids and xylem fibres; tracheal elements show reticulate scalariform and spiral thickenings; xylem fibres elongated with narrow lumen and pointed end; mesocarp contains large aggregates of numerous irregular silica crystals.

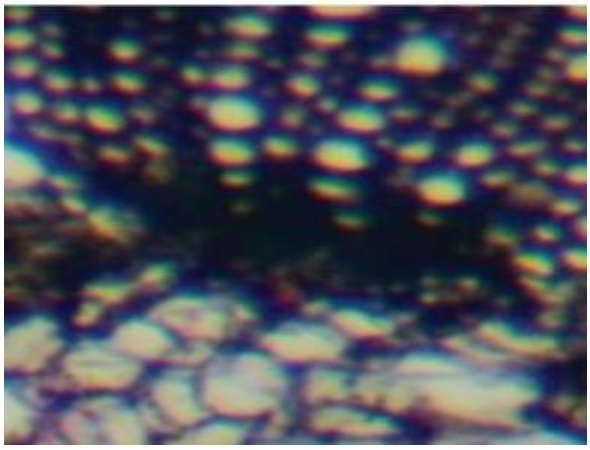


Fig IV. 3 Xylem fibres

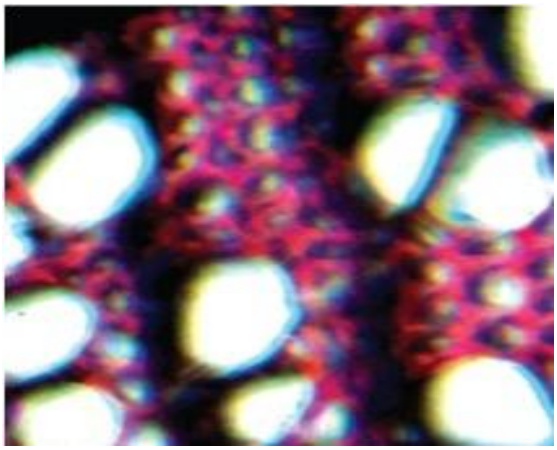


Fig IV. 4 tracheal elements

Powder microscopy:

Fine powder shows Sclereids with uniformly thickened straight walled, isodiametric parenchyma cells with irregular thickened walls, occasionally short fibres and tracheids.

Powder microscopy of *Amalaki* revealed the presence of following microscopical characteristics,

Fig IV.5 sclereid

Fig IV.6 Group of lignified fibres

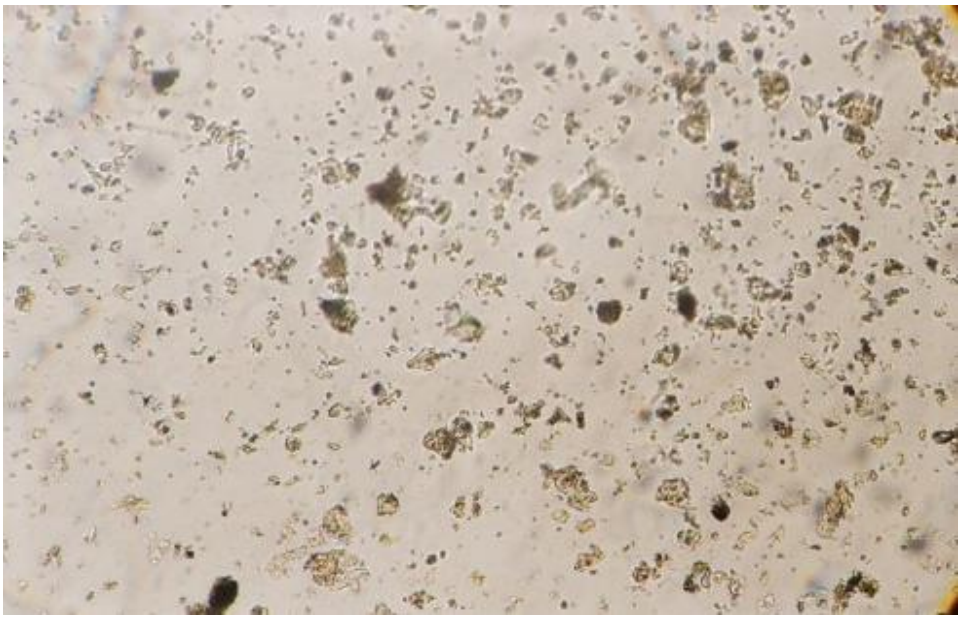


Fig IV. 7 Starch grains

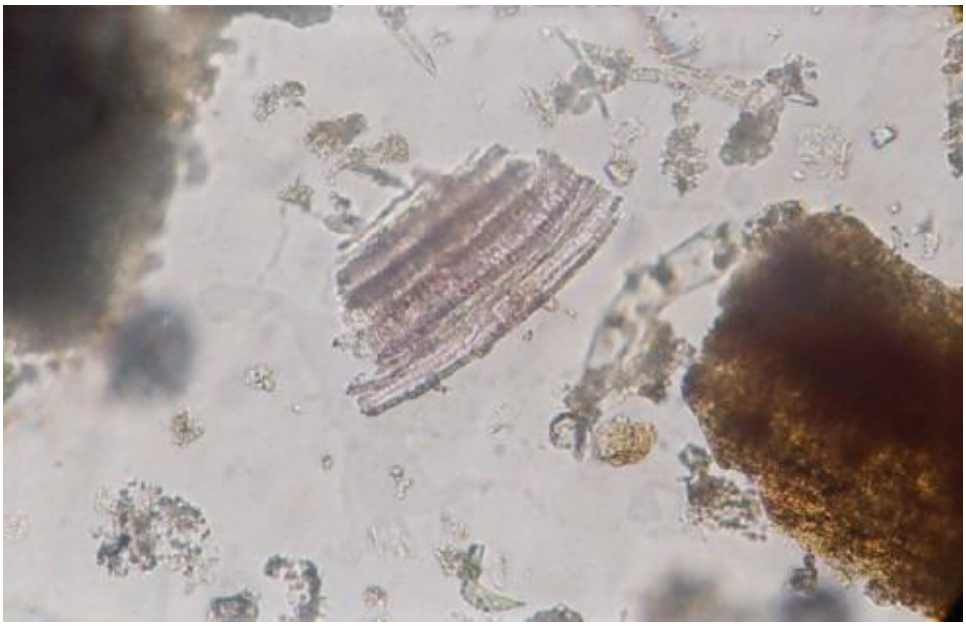


Fig IV.8 Annular vessels

B) *Phyllanthus Amarus*

Microscopical characters

L.S. Structure of the fruit

The fruit when viewed in longitudinal section (LS-VIEW), the fruit appears circular in outline with thick, short lobed stigmatic lobes at the top of the fruit and numbers scattered free seeds. The stigma consists of parenchymatous ground tissue in which thick masses of sclereids (fig.IV.9).

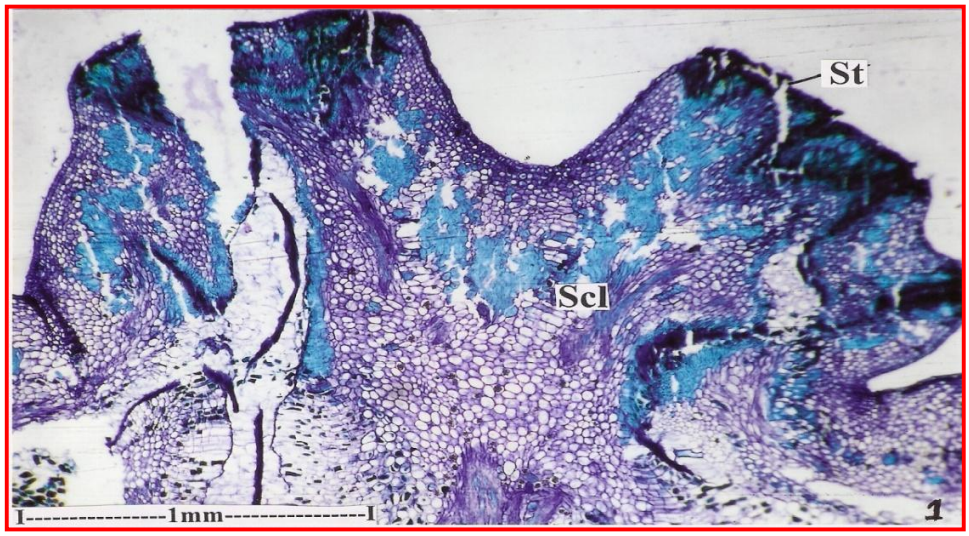


Fig IV.9 L.S of Stigma – Parenchymatous ground tissues and Sclereid masses

(Scl: Sclereids; St: Stigma;) **T.S of the Pericarp**

The pericarp of the berry is 550µm thick. It consists of a thin epidermal layer which represents the epicarp of the pericarp. The epidermal cells are filled with dense, dark tannin (fig IV.8). The major part of the pericarp is the parenchymatous mesocarp. The mesocarp cells circular to polygonal in outline think walled and compact. The inner most layer of the pericarp is disintegrated, small vascular stand are scattered in the mesocarp ground tissue. The vascular strands are less prominent; they consist of small clusters of narrow xylem elements and phloem cells (fig IV.9).

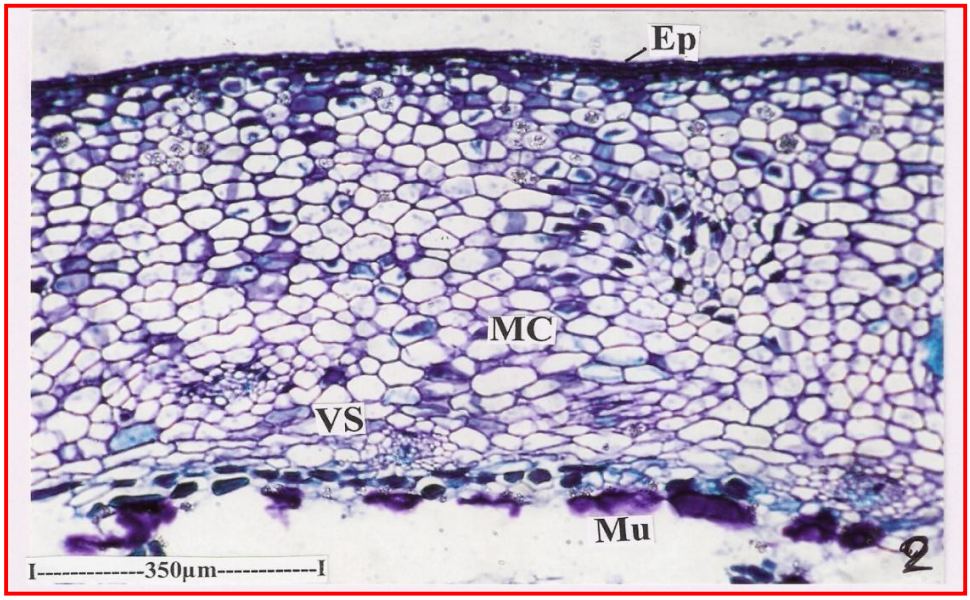


Fig IV.10

T.S of the Pericarp (Fruit wall)
(Ep: Epidermis; Me: Mesocarp; Mu: Mucilage; Vs: Vascular strand)

T.S of the fruit through central part

In transverse sectional view of the berry, a thick central placental tissue is seen from which five thick septa vadiate towards the pericarp (fig IV.9). The placental tissue consists of two wide fan-shaped vascular stands; densely crowded wide, thick walled xylem elements are seen in the vascular stands (fig IV.10).

Fig.IV.11 T.S of the fruit through central part- free central placental Tissue, septa and numerous free seeds

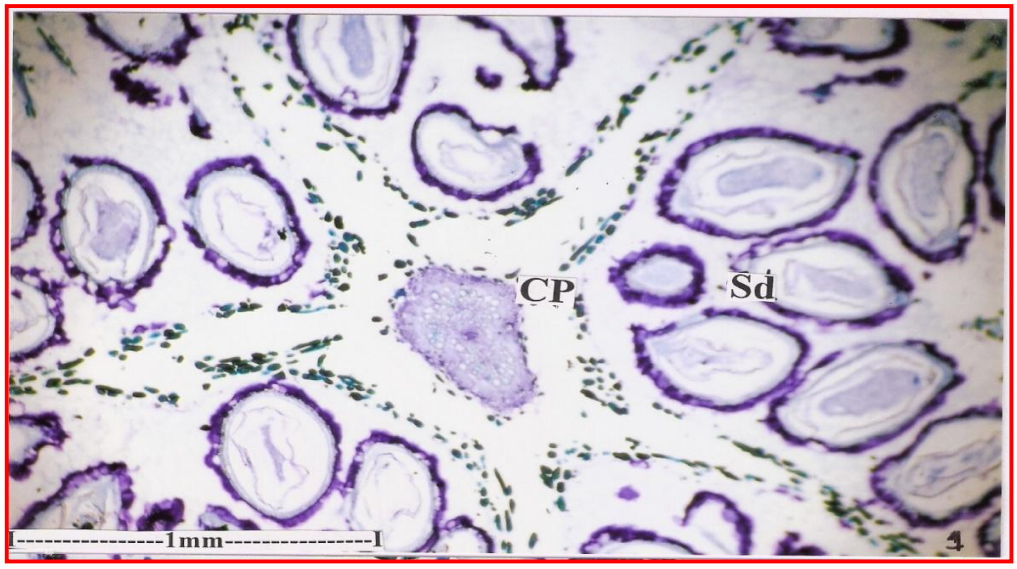
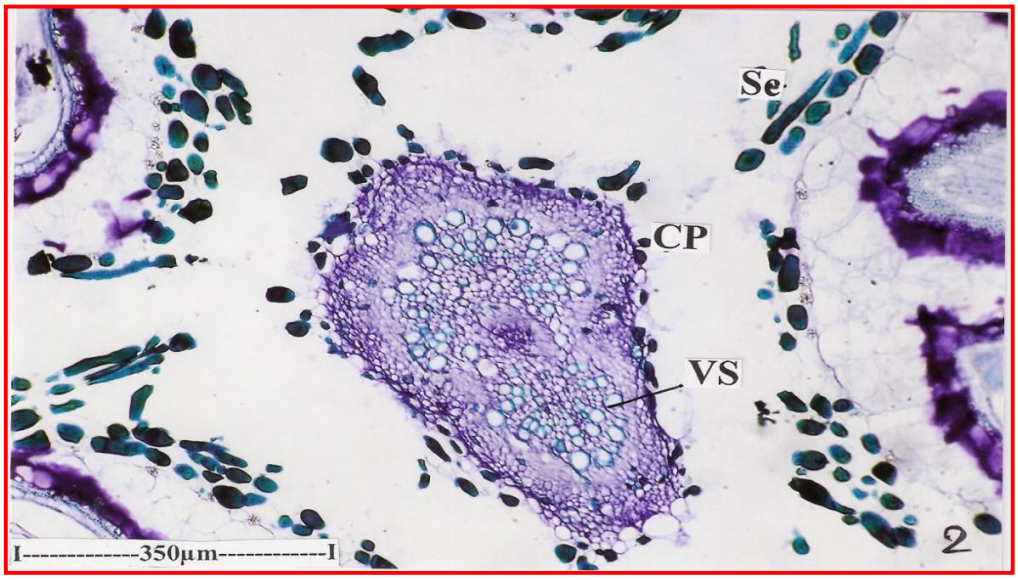


Fig IV.12 Central placentam- Vascular elements



(Cp: Central placentam; Sd: Seeds; Sc: Septum; Vs: Vascular strand)

Structure of the seeds (Fig. IV 13 &14)

The seeds are circular in transactional view (fig.IV.13) and ovate in longitudinal sectional view (fig.IV.14). It has thick mucilage sheath all around. The seeds are 400µm wide and 850µm in length. The mucilage sheath is more or less uniform in thickness (fig. IV.14).

The seed-coat consists of a thin outer layer of elliptical parenchyma cells which seem to secrete the mucilage. Inner to this mucilaginous epidermis is a thick unistratose sclerotic layer lignified walls and wide lumen and stain blue with O'toluidine blue dye (fig. IV.15). The sclerotic layer is 20µm thick.

Fig IV.13 Seeds in transactional view

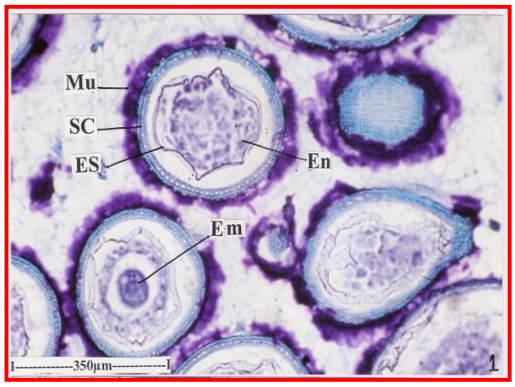


Fig IV .14 A Seed in LS view

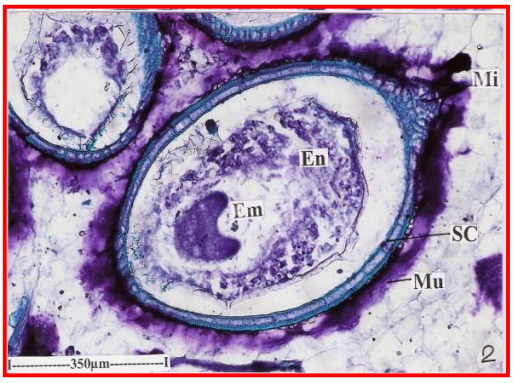
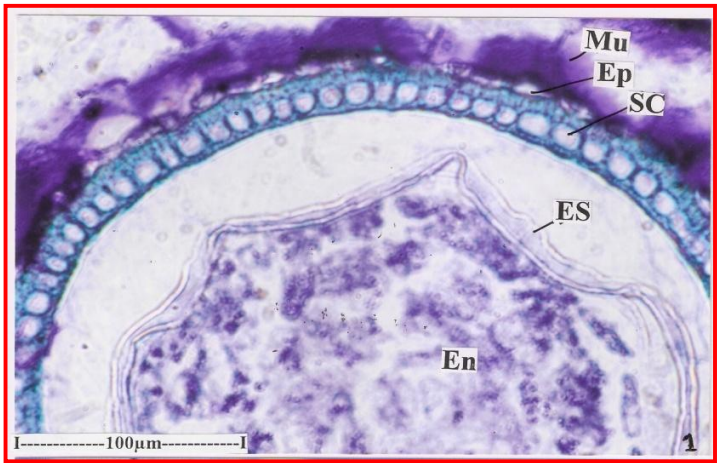


Fig IV.15 T.S of seed coat and Embryo Sac with endosperm



(En: Endosperm; Em: Embryo- Heart shaped stage; Es: Emluyosae;

Mi: Micropylar end of the seed; Mu: Mucilage sheath; Sc: Seed coat)

Powder microscopic characteristics

The powder of the fresh fruits shows the following cell inclusions;

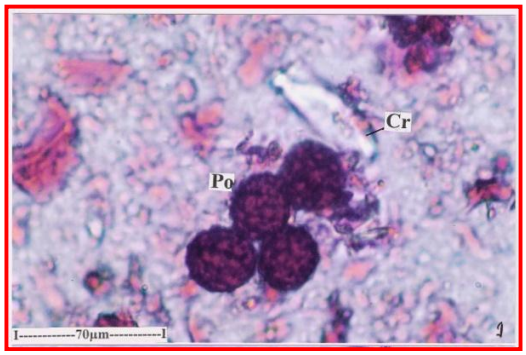
(1) Pollen Grains (fig IV.16 &17)

Pollen grains sticking on the surface of the berries are seen in small clusters in the power. The pollen grains are spherical measuring 50µm in diameter. The exine of the pollen shows reticulate thick endings.

(2) Calcium oxalate crystals (fig IV.18; Fig IV.19).

Calcium oxalate crystals of various shapes are abundant in the powder. They are club shaped, elliptical or rectangular in outline. These crystals occur in the mucilage secreting outer epidermis of the seed coat (fig 14). The elongated crystals are 130µm long and 50µm wide.

Fig IV. 16 A Cluster of pollen grains



FigIV. 17 Two pollen-enlarged Shows the reticulate exine

(Cr: Crystal body; Ex: Exine showing reticulate thickenings; Po: Pollen)

Fig IV.18 Calcium oxalate crystal

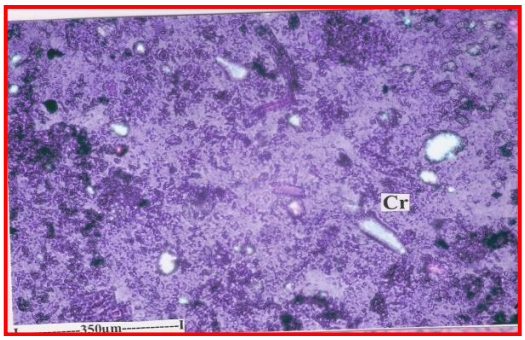
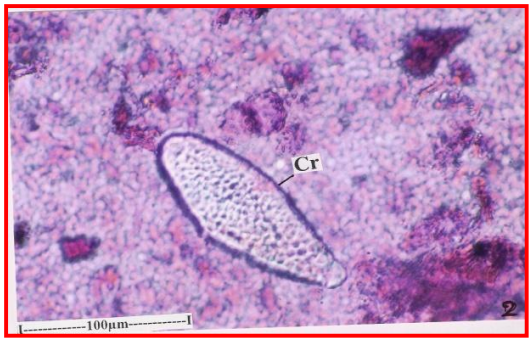


Fig IV. 19 Cute shaped crystal mass, enlarged



(Cr: Crystal

CHAPTER - V

Physico chemical study

PHYSICO CHEMICAL STUDY[15, 21,29,35]

(i) *EXTRACTIVE VALUES*

A) Determination of Petroleum Ether Extractive value :

About 10 gm of the drug powder sample-1, 2&3 are macerated with 100 ml of the Petroleum Ether in a stoppered flask separately for 24 hrs with frequent shaking in a mechanical shaker. Then it is filtered using muslin cloth to the beaker whose weight is already known. Then the filtrates is evaporated to dryness in dryness is a heating mantle using appropriate temperature. Then the weight of beaker with extract is taken the percentage extractive value is calculated with reference to the dried powder.

B Determination of Benzene Extractive value :

About 10 gm of the drug powder sample-1, 2&3 are macerated with 100 ml of the Benzene in a stoppered flask separately for 24 hrs with frequent shaking in a mechanical shaker. Then it is filtered using muslin cloth to the beaker whose weight is already known. Then the filtrates is evaporated to dryness in dryness is a heating mantle using appropriate temperature. Then the weight of beaker with extract is taken the percentage extractive value is calculated with reference to the dried powder.

C) Determination of Chloroform Extractive value :

About 10 gm of the drug powder sample-1, 2&3 are macerated with 100 ml of the Chloroform in a stoppered flask separately for 24 hrs with frequent shaking in a mechanical shaker. Then it is filtered using muslin cloth to the beaker whose weight is already known. Then the filtrates is evaporated to dryness in dryness is a heating mantle using appropriate temperature. Then the weight of beaker with extract is taken the percentage extractive value is calculated with reference to the dried powder.

D) Determination of Methanol Extractive value :

About 10 gm of the drug powder sample-1, 2&3 are macerated with 100 ml of the Methanol in a stoppered flask separately for 24 hrs with frequent shaking in a mechanical shaker. Then it is filtered using muslin cloth to the beaker whose weight is already known. Then the

filtrates is evaporated to dryness, a heating mantle using appropriate temperature . Then the weight of beaker with extract is taken the percentage extractive value is calculated with reference to the dried powder.

E) Determination of Water Extractive value :

About 10 gm of the drug powder sample-1, 2&3 are macerated with 100 ml of the Water in a stoppered flask separately for 24 hrs with frequent shaking in a mechanical shaker. Then it is filtered using muslin cloth to the beaker whose weight is already known. Then the filtrates is evaporated to dryness in dryness is a heating mantle using appropriate temperature. Then the weight of beaker with extract is taken the percentage extractive value is calculated with reference to the dried powder.

(ii) DETERMINATION OF ASH VALUE

A) Determination of Total Ash :

About 2gms (accurately weighed) of the powdered drug was taken in silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red leaf, until free from carbon cooled and weighed. The percentage of ash was calculated with reference to the air dried powder. The procedure was repeated to get constant weight.

B) Determination of Water Soluble Ash :

The total ash was boiled with 25ml of water and filter with (Whatmann no- 4) filter paper. It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and water insoluble matter was weighed. The water soluble ash can is calculated by subtracting the water insoluble matter from the total ash.

C) Determination of Acid insoluble Ash :

The total attained was boiled for 5 min with 25 ml of 10%w/v dilute Hydrochloric acid and filtered through an ash less filter paper was ignited in the silica crucible; cooled acid insoluble ash was weighed.

D) Determination of Sulphated Ash :

About 1gm of the powder was weighed in a silica crucible. The powder was moist with 1ml of concentrated sulphuric acid and adds few drops concentrated sulphuric acid ignited and repeated until the difference of weighed was not more than 0.5gm and calculated the percentage of sulphated ash with reference to air dried drug

(iii) DETERMINATION OF FIBRE CONTENT

Procedure :

3gm of the finally powdered crude drug weighed and extracted with petroleum ether at room temperature. After that boiled with 300ml sulphuric acid for 30 minute. Filter the extract material through a muslin cloth and wash with boiling water. Then boil the material with 200ml sodium hydroxide for 30 minutes. Filter through muslin 25ml of alcohol successively. After washing the residue transfer to silica crucible, which was previously weighed (W1). Dry the residue for 2 to 3 hours for 130^{o} C and cool the crucible in the desiccators and weigh again (W2). Incinerate the residue for 30 minute at 100^{o} C and cool it to room temperature in a desiccator and weigh again (W3). Then calculated by using the following,

(W2-W1) - (W3-W1) / weight of sample x 100

(iv) FLUORESCENCE CHARACTERISTIC OF THE SAMPLE[11,15]

Many crude drugs show fluorescence when the sample is exposing to ultraviolet radiation. Evaluation of crude drugs based on fluorescence in daylight is not of much use as it is usually likely to be unreliable due to the weakness of the fluorescent effect. Fluorescent lamps are fitted with a suitable filter, which eliminates visible radiation from the lamp and transmits ultraviolet radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.

A very important generalization made by stokes in 1852 stated that in fluorescence, the fluorescent light is always of greater wavelength than the exciting light. Light rich in short

wavelength is very active in producing fluorescence so that strong ultraviolet light produces fluorescence in many substance which do not visibly give fluorescence in day light.

The fluorescence studies were done for the powder of the fruits of ***Phyllanthus Emblica*** **Linn.** And ***Phyllanthus Amarus*** as such and also by treating the whole plant powder with different chemical reagents. The fluorescence studies on different solvent extracts of powder of the fruits of ***both the plants*** *were* also performed under daylight and UV-light.

CHAPTER - VI

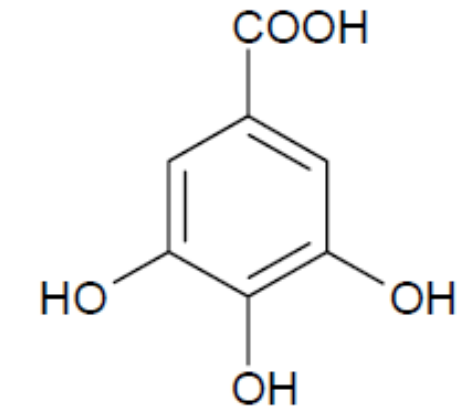
Phytochemical study

PHYTOCHEMICAL STUDY[7,13,19,21]

Phyllanthus emblica

Chemical constituents:

The fruits of ***Emblica officinalis*** are rich in tannins. The fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B, which have antioxidant properties, one on hydrolysis gives gallic acid, ellagic acid and glucose wherein the other gives ellagic acid and glucose. The fruit also contains Phyllemblin1-3.



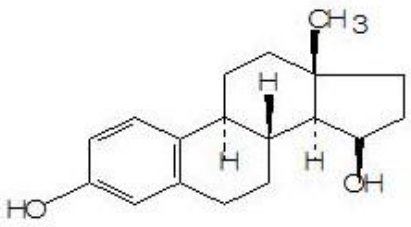
$C_7H_6O_5$ Mol. Wt. 170.12

Gallic acid

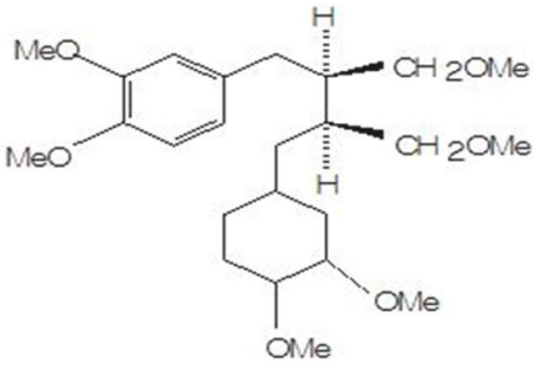
Phyllanthus amarus

Chemical constituents:

It is a rich source of plant chemicals, including many which have been found only in the ***Phyllanthus*** genus. Many of the "active" constituents are attributed to biologically active lignans, glycosides, flavanoids, alkaloids, ellagitannins, and phenylpropanoids found in the leaf, stem, and root of the plant. Common lipids, sterols, and flavanols also occur in the plant. The main plant chemicals in ***Phyllanthus amarus*** include alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, lupeols, methyl salicylate, niranthin, nirtetralin, niruretin, amarusn, amarusne, amarusside, norsecurinines, phyllanthin, phyllanthine, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal, and tricontanol.



Estradiol



Phyllanthin[41]

PHYTOCHEMICAL TESTS[13]

The extract of the plant material leaf was separately prepared and subjected to chemical tests for the identification of its chemical constituents.

(i) TEST FOR ALKALOIDS

A) Mayer's test:

Taken fraction of extract & added Mayer's reagent. It gave cream colour precipitate which indicates the presence of alkaloids.

B) Dragendroff's test:

Taken fraction of extract & added Dragendroff's reagent. It gave reddish colour precipitate which indicates the presence of alkaloids.

C) Wagner's test:

Taken fraction of extract & added Wagner's reagent. It gave reddish brown colour which indicates the presence of alkaloids.

D) Hager's test:

Taken fraction of extract & added Hager's reagent. It gave yellow colour precipitate which indicates the presence of alkaloids.

(ii) TEST FOR CARBOHYDRATES

A) Molisch's test:

Taken fraction of extract and added 2- napthol & few drops of Sulphuric acid through the side of the test tube. Violet ring was observed at the junction of two layers which indicates the presence of carbohydrates.

B) Fehling's Test:

Taken fraction of extract and added Fehling's Solution A&B and heated on water bath for few minutes. Red precipitate of Cuprous Oxide was observed which indicates the presence of carbohydrates.

C) Barfoed's Test:

Taken fraction of extract and added Barfoed's reagent and heated on water bath for few minutes. It gave red precipitate which indicates the presence of carbohydrates.

(iii) TEST FOR PROTEINS

A) Ninhydrin test:

Taken fraction of extract and added Ninhydrin reagent. Purple colour appeared and it indicates the presence of Proteins.

B) Biuret Test:

Taken fraction of extract and added Sodium hydroxide and Copper sulphate. Violet colour appeared and it indicates the presence of Proteins.

C) Xantho Protein Test:

Sample Solution was mixed with equivalent quantity of concentrated Nitric acid and boiled. Made it alkaline with Sodium hydroxide solution. Yellow colour changed to deep yellow to orange which indicates the presence of Proteins.

(iv) TEST FOR TANNINS AND PHENOLIC COMPOUNDS

A) Ferric chloride test:

Taken fraction of extract and mixed with Neutral Ferric chloride Solution. Brownish colour was obtained which indicates the presence of Tannins and Phenolic Compounds.

B) Lead acetate Test:

Taken fraction of extract and added 10% Lead acetate Solution. White Precipitate obtained was which indicates the presence of Tannins and Phenolic Compounds.

C) Gelatin Solution Test:

Taken fraction of extract and added 1% Solution of Gelatin containing Sodium Chloride solution. White Precipitate was obtained which indicates the presence of Tannins and Phenolic Compounds.

(v) IDENTIFICATION TEST FOR STEROID GLYCOSIDES

A) Libermann burcher Test:

The extract was dissolved in chloroform and with the chloroform extract added few ml of acetic anhydride and one or two drops of concentrated sulphuric acid were added to the above solution. It changed colour from blue, violet and to green. It indicates the presence of steroidal glycosides.

(vi) TEST FOR FLAVANOIDS

A) Shinoda test :

Taken small quantity of extract and added Mg/HCL which gave yellow to red colour. It indicates the presence of flavanoids.

- Taken small quantity of extract and added Ammonium solution which gave red colour. It indicates the presence of flavanoids.
- Taken small quantity of extract and added ethyl amino ester & dissolved in Poly ethylene glycol. Observed under UV light, which showed yellow to orange fluorescence. It indicates the presence of flavanoids.

CHROMATOGRAPHICAL EVALUATION[11,21,22]

Thin layer chromatography[11.22]

- TLC is a very effective technique for the separation of chemical constituent of chemical constituent of an extracted and for their identification.
- TLC profiles developed for an extract from a defined solvent system and other parameters could be used as a finger prints is comparative qualitative evaluation of herbal drugs. The tend of evaluation by this method is becoming popular in view of its simplicity and reproducibility.
- TLC is an important analytical tool in the separation, identification and estimation of different classes of natural products. In this technique, the different components an separated by the differentially migration of solute between two phases a stationary phase and a mobile phase. Here, the principle of separation is adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phase, its preparation and use with different solvent, separation can be achieved on the basis of partition or a combination of partition and adsorption.

(a) Separation of components :

The extract was dissolved in respective solvents separately and spotted using a calibrated capillary tube on a prepared TLC plate 1 cm above from the bottom of the plate. The spot were equally sized and had a diameter ranging from 2-3 mm.

(b) Selection of mobile phase :

The selection of solvent or mobile phase depends upon various factors as mentioned below.

1) Nature of substance to be separated.
2) Nature of stationary phase (Polar/ Non polar).
3) Mode of chromatography (normal / reverse phase).

4) Extent of separation to be achieved (analytical / preparative).

The selection of solvent systems was based on increasing order of polarity. Based on the chemical tests and nature of phytoconstituents present, the solvent systems were selected. The different spots developed in each system were detected by means of specific reagents and iodine staining. The following solvent systems were used to detect some of the phytoconstituents in the extracts.

(C) Stationary Phase :

Precoated TLC plates of E- Merck were used for the study.

HIGH PERFOMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)[18,21]

HPTLC is a modern chromatographic technique in which the principle of TLC is sophisticated and automated by which the samples are accurately utilized for both qualitative and quantitative purpose.

Characteristics of HPTLC layer:

- Thinner 0.2 mm.
- Smaller grain size (7 mm) and closer distribution.
- Better separation performance over a 50% shorter optimum separation distance (50 mm).
- Optical properties ensure a better signal to noise ratio in densitometric evaluation.
- On HPTLC layer, chromatography takes place in the fastest capillary flow range of mobile phase.

Merits of HPTLC over conventional TLC:

- Faster Separation.
- Reduced diffusion, hence improved separation efficiency.
- Detection (Determination) limits lower by a factor of 10-15.

General procedure of HPTLC

- **Preparation of sample** :

Preparation of sample and standard is an important tool in HPTLC analysis. Sample should be prepared in a solution form with suitable solvents in proper concentration. The selection of solvents depends on the type of solvent used for extraction.

- **Application of sample :**

Commercially available precoated HPTLC plates (Merck) can be used for the study. The solutions of various concentrations should be applied on the respective HPTLC plates using Linomat IV applicator. The plates were dried after application.

- **Development of plates :**

Plates should be developed in a suitable solvent system to the premarked distance. These plates should be removed from the chamber, dried and then scanned.

- **Detection :**

The developed plates should be observed under day light and UV light for the detection of constituents.

- **Densitometric Scanning :**

The developed plates should be scanned at a suitable wavelength for the quantitative and qualitative analysis. Peak areas and peak heights should be recorded from which unknown concentration of the samples can be determined and compared with the standards.

Preparation of sample :

Accurately weighed about 100 mg of sample extracts of ***Phyllanthus emblica*** *and* ***Phyllanthus Amaru****s* was dissolved in 10ml methanol in a 50ml beaker with stirring for few mts. The solution was filtered through whatmann filter paper and transferred into 10ml flask.The sample solution thus prepared was for HPTLC analysis.

Preparation of standard:

Accurately weighed about 2mg of standard drug was dissolved in 2ml of methanol and stir well for few minutes. The solutions thus prepared were used for HPTLC.

Sr No	Standard marker Used	Extracts
1. **2.**	Gallic acid Methyl salicylate	***Phyllanthus emblica*** ***Phyllanthus amarus***

Application of Sample:

The extracts and the standard were applied on the HPTLC plate using LINOMAT IV APPLICATOR.

Solvent System:-

All the solvent system used for HPTLC was of HPLC grade.

Solvent System (1) Chloroform: tri ethyl amine (9:1)

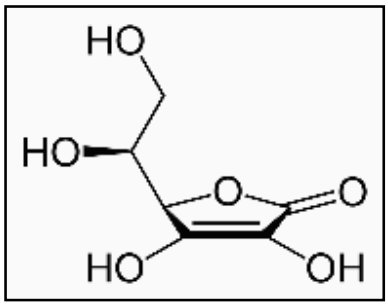
Solvent System (2) Toluene: Ethyl acetate (9.3:0.7)

Detection:

The plate developed and dried and the plates were observed under UV and scanned at wavelength of 254 nm and 365nm.

VITAMIN C ESTIMATION [25, 26]

Vitamin C determination
By Iodine Titration



PRINCIPLE

Vitamin C (ascorbic acid) is an antioxidant that is essential for human nutrition. Vitamin C deficiency can lead to a disease called scurvy, which is characterized by abnormalities in the bones and teeth. Many fruits and vegetables contain vitamin C, but cooking destroys the vitamin, so raw citrus fruits and their juices are the main source of ascorbic acid for most people.

One way to determine the amount of vitamin C in food is to use a redox titration. The redox reaction is better than an acid-base titration since there are additional acids in a juice, but few of them interfere with the oxidation of ascorbic acid by iodine.

Iodine is relatively insoluble, but this can be improved by complexing the iodine with iodide to form triiodide.

$I_2 + I^- \ <—> \ I_3^-$

Triiodide oxidizes vitamin C to form dehydroascorbic acid

$$C_6H_8O_6 + I_3^- + H_2O \text{ --> } C_6H_6O_6 + 3I^- + 2H^+$$

As long as vitamin C is present in the solution, the triiodide is converted to the iodide ion very quickly. However, when the all the vitamin C is oxidized, iodine and triiodide will be present, which react with starch to form a blue-black complex. The blue-black color is the endpoint of the titration.

This titration procedure is appropriate for testing the amount of vitamin C in vitamin C tablets, juices, and fresh, frozen, or packaged fruits and vegetables. The titration can be performed using just iodine solution and not iodate, but the iodate solution is more stable and gives a more accurate result.

Procedure for Determining Vitamin C

1% Starch Indicator Solution :

Add 0.50 g soluble starch to 50 ml boiling distilled water.

Mix well and allow cooling before use. (Does not have to be 1%; 0.5% is fine)

Iodine Solution:

- Dissolve 5.00 g potassium iodide (KI) and 0.268 g potassium iodate (KIO_3) in 200 ml of distilled water.
- Add 30 ml of 3 M sulfuric acid.
- Pour this solution into a 500 ml graduated cylinder and dilute it to a final volume of 500 ml with distilled water.
- Mix the solution.
- Transfer the solution to a 600 ml beaker. Label the beaker as iodine solution.

Vitamin C Standard Solution:

Dissolve 0.250 g vitamin C (ascorbic acid) in 100 ml distilled water.

Dilute to 250 ml with distilled water in a volumetric flask. Label the flask as vitamin C standard solution.

Titration procedure

Standard:

Add 25.00 ml of vitamin C standard solution to a 125 ml Erlenmeyer flask. Add 10 drops of 1% starch solution. Titrate the solution until the endpoint is reached. The end point will be the sign of blue color that persists after 20 seconds of swirling the solution. Record the final volume of iodine solution. Repeat the titration at least twice more.

Samples:

Add 25.00 ml of juice sample to a 125 ml Erlenmeyer flask. Add 10 drops of 1% starch solution. Titrate until the endpoint is reached. (Add iodine solution until you get a color that persists longer than 20 seconds.) Repeat the titration at least twice more.

IN VITRO ANTI-OXIDANT PROPERTY SCREENING [2, 7, 14, 19,227,28,33,40,41]

INTRODUCTION

Plant and plant products are being used as a source of medicine since long. Among the most important constituents of edible plant products, low molecular weight antioxidants are the most important species. It is known that consumption of fruits and vegetables is essential for normal health of human beings. Vegetarian diet can reduce the risk of cancer, atherosclerosis, etc. *Phyllanthus emblica, Phyllanthus amarus,* has been used in Ayurveda, the ancient Indian system of medicine. It has been used for treatment of several disorders such as common cold, scurvy, cancer and heart diseases. It is believed that the major constituent responsible for these activities is vitamin C (ascorbic acid). Ascorbic acid shows antioxidant, anti-inflammatory and antimutagenic properties. It is a very effective free-radical scavenger.

Scavenging of hydrogen peroxide radicals

Chemicals used

Hydrogen peroxide: 0.2267ml of Hydrogen peroxide is diluted in 100ml PBS.

Preparation of Test and Standard Solutions:

25µg, 50µg, upto 250µg/ml, alcoholic extract of each powdered drug (including standard epicatechin and ascorbic acid) was prepared by using solvent ethanol. Then these dilutions are used for following method.

Methodology:

- A solution of hydrogen peroxide (20mMol/l) was prepared in phosphate buffer saline (PBS) pH 7.4.

- Various concentration of 1ml of the extract or Standard in methanol was added to 2ml of hydrogen peroxide solution in PBS.
- The absorbance was measured at 230 nm, after 10mts against a blank solution that contained extract in PBS without hydrogen peroxide.

DPPH FREE RADICAL SCAVENGING ACTIVITY[2, 14, 19,27,33,41]

The free radical scavenging activity of plant extract was measured by the decrease in absorbance of methanolic solution of DPPH. A stock solution of DPPH (33 mg L) was prepared in methanol and the 5 ml of this stock solution was added to 1 mL of the plant extract solution at different concentration (250, 500, 1000, 1500, 2000, 2500 µg/ml). After 30 minute, absorbance was measured at 517nm and compared with the standards, i.e , ascorbic acid, (10-50µg mL). Scavenging activity was expressed as the percentage inhibition.

Percentage of inhibition= [(A control – A sample)/A control] X 100%

CHAPTER - VII

PHARMACOLOGICAL ACTIVITY

PHARMACOLOGICAL ACTIVITY

ANTIEPILEPTIC ACTIVITY

a. Maximal electroshock induced convulsion[43]

Procedure

Seizures are induced to all the groups by using a Electroconvulsiometer. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increased the contact and reduced the incidence of facilities. Different doses of the MERR were administered for 14 days before induction of seizures. The duration of various phases of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition of Hind Limb Tonic Extension[100] (HLTE)**.**

Group I Animals treated with 1% SCMC, 1ml/100g

Group II Animals treated with phenytoIn (25mg/kg) suspended in 1% w/v SCMC

Group III Animals treated with MEPE (200mg/kg) suspended in 1% w/v SCMC

Group IV Animals treated with MEPN (200mg/kg) suspended in 1% w/v SCMC

b. Determination of the effect of Rubus racemosus and on neurotransmitter concentrations in rat brain after induction of epilepsy

The various biogenic amines in discrete regions of the rat brain were estimated by spectroflurorimetric method.

Reagents

1) HCl-Butanol – 0.85ml of 37% HCl was added to one liter of butanol to get HCl-butanol solution
2) Heptane
3) 0.4M HCl
4) EDTA (pH 6.9)
5) 0.1 M Iodine

6) Sodium thiosulphate solution

7) 5 M Sodium hydroxide

8) 10 M acetic acid

9) Dopamine Standard

10) Nor-adrenaline Standard

11) 0.1M HCl

12) o-pthaldialdehyde reagent

PROCEDURE

Preparation of Tissue Extracts[44]

Dissected frozen rat brains were first cut on a cooled microtome (-200) in to frontal slices (about 1mm thick) at pre determined antero posterior levels. The frontal slices were subsequently placed on the cooled stage (-200) of a punching apparatus where cylindrical tissue samples (usually 1 mm in diameter, same thickness as the slice) were punched out of selected brain areas with a glass tube. The x and y co-ordinates of the center of the area were adjusted is a stereo microscope, ocular of which contained cross line that were concentric with the center of the glass tube. For weight determination the tissue pieces were transferred immediately to pre-cooled micro homogenizers which were closed with glass stored at –250.

Extraction

The tissue was homogenized in 0.1ml HCl-Butanol for 1 minute in a glass homogenizer made from a small centrifuge tube (vol. 1.5ml) the total volume was considered to give 0.105ml, taking account of the tissue volume (1mg = 0.001ml) the sample was then centrifuged for 10 min at 2000 rpm. An aliquot of the supernatant phase (0.8ml) was added to an eppendroff reagent tube containing 0.2ml heptane (for spectroscopy) and 0.025ml HCl 0.1M. After 10 min of vigorous shaking, the tube was centrifuged under the same condition as above in order to separate the two phases and the overlaying organic phase was discarded, the aqueous phase (0.02ml) was then taken either for a 5-HT or NA and DA assay. All steps carried at 00C.

Serotonin Assay[45]

As mentioned earlier some modifications in reagent concentration became necessary together with changes in proportions of the solvent, in order to obtain in a good fluorescence yield with reduced volume for 5-HT determination, when o-pthaldialdehyde (OPT) method was employed. Added 0.025ml of OPT reagent to 0.02ml of the HCl extract. The fluorophore was developed by heating to 100oC for 10min. After the samples reached equilibrium with the ambient temperature, excitation and emission spectra of intensity reading at 360 and 470nm were recorded.

Nor-Adrenaline and Dopamine assay[46]

To 0.02ml of HCl phase, 0.005ml 0.4M HCl and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution for oxidation. The reaction was stopped after two minutes by the addition of 0.01ml sodium thiosulphate in 5 M sodium hydroxide and 10 M acetic acid was added 1.5 minutes later. The solution was then heated to 1000C for 6 minutes.

When the sample again reached room temperature, excitation and emission spectra were read (330 and 375 nm for Dopamine and 395 – 485 nm for Nor adrenaline) in a spectrofluorimeter compared the tissue values (fluorescence of tissue extract minus fluorescence of tissue blank) with an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank). Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine). Internal reagent standards were obtained by adding 0.005ml distilled water and 0.1 ml HCl Butanol to 20 mg of dopamine and Nor-adrenaline standard.

CHAPTER -VIII

Results and Discussion

RESULTS AND DISCUSSION

(A) Extractive value

The extractive values of two powdered drug samples S1&S2 were calculated with reference to the weight of air dried powdered drug and the values are given in table no.1.

Table No. 1

SNO.	Solvent	Extraction value %w/w	
		S1	S2
(1)	Petroleum Ether	0.3	2.8
(2)	Benzene	13.1	14.9
(3)	Chloroform	1.3	0.8
(4)	Methanol	24	16
(5)	Water	4.6	5.2

S1- ***Phyllanthus emblica*** **S2-** ***Phyllanthus amarus***

(B) Ash value

The ash values of the two powdered drug samples S1 & S2 were calculated with reference to the weight of air dried powdered drug and the values are given in Table No.2.

Table No.2

SR NO.	Physico-chemical Factors	Value in percentage W/W	
		S1	S2
(1)	Total Ash	4.6	5.4
(2)	Acid insoluble ash	2.8	1.3
(3)	Water insoluble Ash	1.2	1.5
(4)	Sulphated Ash	3.5	2.4

S1- ***Phyllanthus emblica*** S2- ***Phyllanthus amarus***

(C) Fibre content

The Fiber content of two powdered drug samples S1 & S2 were calculated with reference to the equation given in the methodology and the values are given in Table No.3.

Table No.3

Sample	Fibre content
Phyllanthus emblica	0.103%w/w
Phyllanthus amarus	0.416%w/w

Table No.4

Fluorescence analysis of powdered fruits of *Phyllanthus emblica* Linn.

S.NO.	TREATMENT	DAY LIGHT	UV LIGHT
			254nm
1.	**Powder as such**	Dark Brown	Brown
2.	**Water**	Light brown	Light Green
3.	**5% NaOH**	Green	Light Yellow
4.	**1N NaOH**	Light Green	Yellowish Green
5.	**1N HCl**	Pale Yellow	Brown
6.	**50% H_2SO_4**	Brown	Light Brown
7.	**50% HNO_3**	Yellowish Brown	Light Yellow
8.	**Picric Acid**	Yellow	----------
9.	**Acetic acid**	Light brown	Light brown
10.	**Ferric chloride**	Light Green	Dark yellow
11.	**HNO_3 + NH_3**	Brown	Light brown

Table No.5

Fluorescence analysis of powdered fruits of *Phyllanthus amarus* Linn.

S.NO.	TREATMENT	DAY LIGHT	UV LIGHT
			254nm
1.	**Powder as such**	Light Brown	----------
2.	**Water**	Light brown	Light Green
3.	**5% NaOH**	Green	Light Yellow
4.	**1N NaOH**	Light Green	Yellowish Green
5.	**1N HCl**	Pale Yellow	Brown
6.	**50% H_2SO_4**	Brown	Light Brown
7.	**50% HNO_3**	Yellowish Brown	----------
8.	**Picric Acid**	Yellow	Brown
9.	**Acetic acid**	Light brown	Light brown
10.	**Ferric chloride**	Light Green	----------
11.	**HNO_3 + NH_3**	Brown	Light brown

(D) Phytochemical test

Qualitative phyto-chemical analysis of ***phyllanthus emblica, Phyllanthus amarus*** extracts showed the presence of majority of the compounds including alkaloids, carbohydrates, proteins & free amino acids, tannins &phenolic compounds, steroidal glycosides, and flavanoids.

Table No.6

SR NO.	CHEMICAL TEST	METHANOLIC EXTRACTS	
		Phyllanthus emblica	***Phyllanthus amarus***
(1)	**Alkaloids**		
A	Mayer's test	+	+
B	Dragon droff's test	+	+
C	Wagner's test	+	+
D	Hager's test	+	+
(2)	**Carbohydrates**		
A	Molisch's test	+	+
B	Fehling's test	+	+
C	Barfoed's test	+	+
(3)	**Proteins and Free Amino acids**		
A	Ninhydrin test	+	+
B	Biuret test	+	+
C	Xantho Protein test	+	+
(4)	**Tannins and Phenolic Compounds**		
A	Ferric chloride test	+	+
B	Lead acetate test	+	+
C	Gelatin Solution	-	-
(5)	**Steroidal Glycosides**		
A	Libermann burcher test	+	+
(6)	**Flavanoids**		
A	Shinoda test	+	+
B	Extract + Ammonia	+	+
C	Extract + Ethyl acetate	+	+

"+" indicates presence, "-'' indicates absence

(E) HPTLC FINGERPRINTS

Table No.7

HPTLC analysis of plant extracts

Sr. No	Particulars	No of Peaks	RF Value	Area	Area %
1)	Methanolic extract of ***Phyllanthus emblica***	5	0.10, 0.34,0.75,1.16,**1.32**	6271.7	23.97%
2)	Methanolic extract of ***Phyllanthus amarus***	5	0.12,0.24,0.37,0.50,**1.29**	11932.4	43.13%
3)	Gallic acid	4	0.14, 0.23, 0.95 , **1.37**	5253.1	77.17%
4)	Methyl salicylate	1	**0.12**	6019.1	100%

Quantitative estimation of Standard (Ascorbic Acid) Components in the plant extract

1) Gallic acid in methanolic extract of ***Phyllanthus amarus :*** 2.796µg of standard
2) Gallic acid in methanolic extract of ***Phyllathus emblica***: 1.553 µg of standard

Quantitative estimation of Standard (Epicatechin) Components in the plant extract

1) Methylsalicylate in methanolic extract of ***Phyllathus emblica*****:** 3.476µg of standard
2) Methylsalicylate in methanolic extract of ***Phyllanthus amarus*** : 2.243µg of standard

(F) ESTIMATION OF VITAMIN C

The quantities of vitamin c present in the plant samples were calculated by using the following formula. Quantity of vitamin c present in the sample = Standard value / 0.25gm vitamin C = sample titre value / x ml vitamin C. The quantities of the vitamin C, present in the given samples are given in Table No.6.

Estimation of vitamin C

Table No.8

Sr No.	Plant extract	Amount of vitamin C
1	***Phyllathus emblica***	0.68gm/25ml
2	***Phyllanthus amarus***	0.52 gm/25ml

(G) ESTIMATION OF ANTIOXIDANT ACTIVITY

Free radicals, mainly the reactive oxygen species (ROS), involved in initiation, promotion, and progress ion of carcinogenesis. Reactive oxygen species induce oxidative damage of DNA and Cellular components leading to cancer related mutations. Consequently, antioxidant play an important role in the protection of human body, against damage by reactive oxygen species and also the intake of natural anti oxidant has been associated with reduced risk of cancer and other diseases related with oxidative damages.

The two plants are having antioxidant activity (The DPPH scavenging activity of these plants), is related to the phenolic compound and the phenolic hydroxyl group.

The concentration of hydrogen peroxide in water varies according to the phenolic compound. Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of $H_2O_2 \rightarrow H_2O$.

Reactive oxygen species (ROS) including free radicals such as super oxide anion radicals, hydroxyl radicals, non free radicals such as H_2O_2, singlet oxygen along with various forms of active oxygen are involved in various physico chemical processes in the body and aging.

Among all the two extracts tested, Amla (fruit) extract showed potent antioxidant activity with IC 50 value of 35.03µg/ml.

HYDROGEN PEROXIDE METHOD

Hydrogen peroxide is mainly produced by enzymatic reaction. These enzymes are located in microsomes, peroxysomes and mitochondria. In plant and animals cells super oxide dismutase is able to produce hydrogen peroxide by dismutation of oxygen, thus contributing to the lowering of oxidative reactions. The natural combination of dismutase and catalase contributes to remove hydrogen peroxide and thus has a true cellular anti oxidant activity. Hydrogen peroxide is also able to diffuse easily through cell membrane. The generation of hydrogen peroxide by activated phagocytes is known to play an important part in the killing of several bacterial and fungal strains.

The ability of the plant extracts to scavenge hydrogen peroxide is followed by decay in hydrogen peroxide concentration.

$$O^{2-} + H_2O_2 \rightarrow OH^- + OH^+ + O_2$$

.All the samples are given in series according to their IC_{50} value.

The scavenging ability of various plant extract with hydrogen peroxide is shown in table no. 1,2,3,4 & 5 and fig. 5,6,7,8 & 9 and compared with the standard ascorbic acid and epicatechin. It is noticed that all the extracts are capable of scavenging hydrogen peroxide in an amount dependent manner.

The $I.C_{50}$ of scavenging hydrogen peroxide was determined and found to be as follows:

S1- 64.00 µg/ml

S2- 55.00 µg/ml

It was compared with the standard Gallic acid $I.C_{50}$ value (38 µg/ml) and epicatechin (70 µg/ml).

The result reveals that all the extracts have the scavenging character in accordance with the standards. They are in the following trends,

Phyllanthus emblica* < *Phyllanthus amarus

The result of free radicals scavenging activity of ethanolic extract of the two plants and the positive control ascorbic acid, and epicatechin in HYDROGEN PEROXIDE free radical system was shown in table 1, 2, 3, & 4.

Among all the two extracts tested, ***Phyllanthus amarus*** extract showed potent antioxidant activity with IC_{50} value of 55 µg/ml.

Phyllanthus emblica

Table No.9

Sr. No.	Concentration (µg/ml)	Absorbance			Average	% Inhibition
		1	2	3		
1	25	0.378	0.379	0.378	0.378	31.02
2	50	0.356	0.354	0.357	0.355	35.03
3	75	0.210	0.211	0.212	0.211	61.67
4	100	0.202	0.205	0.203	0.203	62.95
5	125	0.189	0.190	0.189	0.189	65.51
6	150	0.185	0.187	0.185	0.185	66.24
7	175	0.181	0.182	0.181	0.181	66.97
8	200	0.174	0.176	0.175	0.175	68.06
9	225	0.170	0.172	0.171	0.171	68.97
10	250	0.170	0.169	0.168	0.169	69.16

Phyllanthus amarus

Table No.10

Sr. No.	Concentration (µg/ml)	Absorbance			Average	% Inhibition
		1	2	3		
1	25	0.430	0.431	0.432	0.431	21.35
2	50	0.196	0.198	0.196	0.196	64.23
3	75	0.189	0.188	0.190	0.189	65.51
4	100	0.174	0.176	0.175	0.175	68.06
5	125	0.171	0.173	0.172	0.172	68.61
6	150	0.165	0.167	0.166	0.166	69.70
7	175	0.161	0.163	0.161	0.161	70.62
8	200	0.159	0.160	0.159	0.159	70.98
9	225	0.159	0.158	0.159	0.159	70.98
10	250	0.150	0.151	0.150	0.150	72.62

Methyl salicylate

Table No.11

Sr. No.	Concentration (µg/ml)	Absorbance			Average	% Inhibition
		1	2	3		
1	25	0.370	0.371	0.370	0.370	32.48
2	50	0.348	0.349	0.348	0.348	36.59
3	75	0.181	0.183	0.181	0.181	66.97
4	100	0.176	0.176	0.177	0.176	67.88
5	125	0.172	0.174	0.173	0.173	68.43
6	150	0.172	0.171	0.172	0.172	68.61
7	175	0.171	0.170	0.171	0.171	68.79
8	200	0.166	0.165	0.166	0.166	69.70
9	225	0.165	0.164	0.164	0.164	70.07
10	250	0.164	0.163	0.164	0.164	70.07

Gallic acid

Table No.12

Sr. No.	Concentration (µg/ml)	Absorbance			Average	% Inhibition
		1	2	3		
1	25	0.359	0.359	0.358	0.359	34.48
2	50	0.212	0.211	0.212	0.212	61.31
3	75	0.210	0.209	0.208	0.209	61.86
4	100	0.204	0.204	0.203	0.204	62.77
5	125	0.205	0.204	0.203	0.204	62.77
6	150	0.200	0.201	0.199	0.200	63.50
7	175	0.191	0.191	0.190	0.191	65.14
8	200	0.189	0.188	0.189	0.189	65.51
9	225	0.185	0.186	0.187	0.186	66.05
10	250	0.139	0.138	0.138	0.138	74.81

Table No.13

Phyllathus emblica	
Concentration µg/ml	% inhibition
0	
25	31.02
50	35.03
75	61.67
100	62.95
125	65.51
150	66.24
175	66.97
200	68.06
225	68.97
250	69.16

Table No. 14

Phyllanthus amarus	
concentration µg/ml	% inhibition
0	0.00
25	21.35
50	64.23
75	65.51
100	68.06
125	68.61
150	69.70
175	70.62
200	70.98
225	70.98
250	72.62

Table No.15

METHYL SALICYLATE (STANDARD)	
Concemration µg/ml	% inhibition
0	0
25	32.48
50	36.59
75	66.97
100	67.88
125	68.43
150	68.61
175	68.79
200	69.70
225	70.07
250	70.07

Table No.16

GALLIC ACID (STANDARD)	
Concemration µg/ml	% inhibition
0	0
25	34.48
50	61.31
75	61.86
100	62.77
125	62.77
150	63.50
175	65.14
200	65.51
225	66.05
250	74.81

The I.C 50 of various species by hydrogen peroxide system were found to be as follows,

Table No .17

Samples	IC 50
Phyllathus emblica	64 µg/mi
Phyllanthus amarus	55µg/mi

Hydrogen peroxide method of *Phyllanthus emblica*

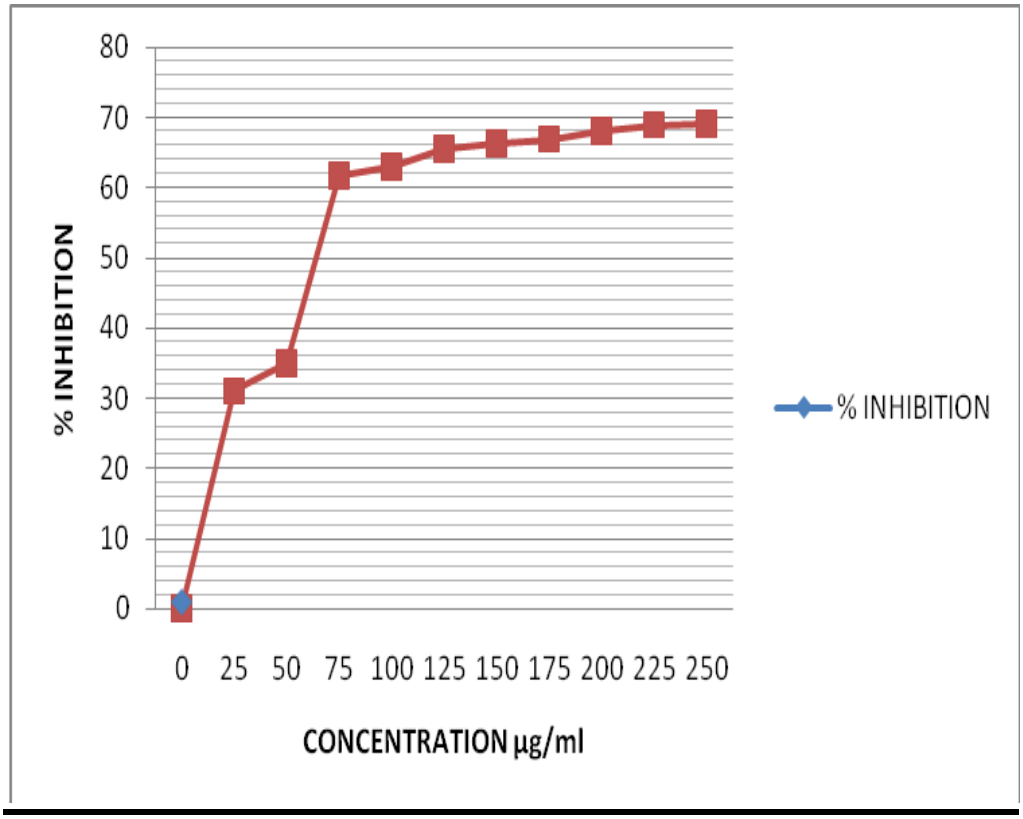


Fig VIII. 1

Hydrogen peroxide method of Phyllanthus amarus

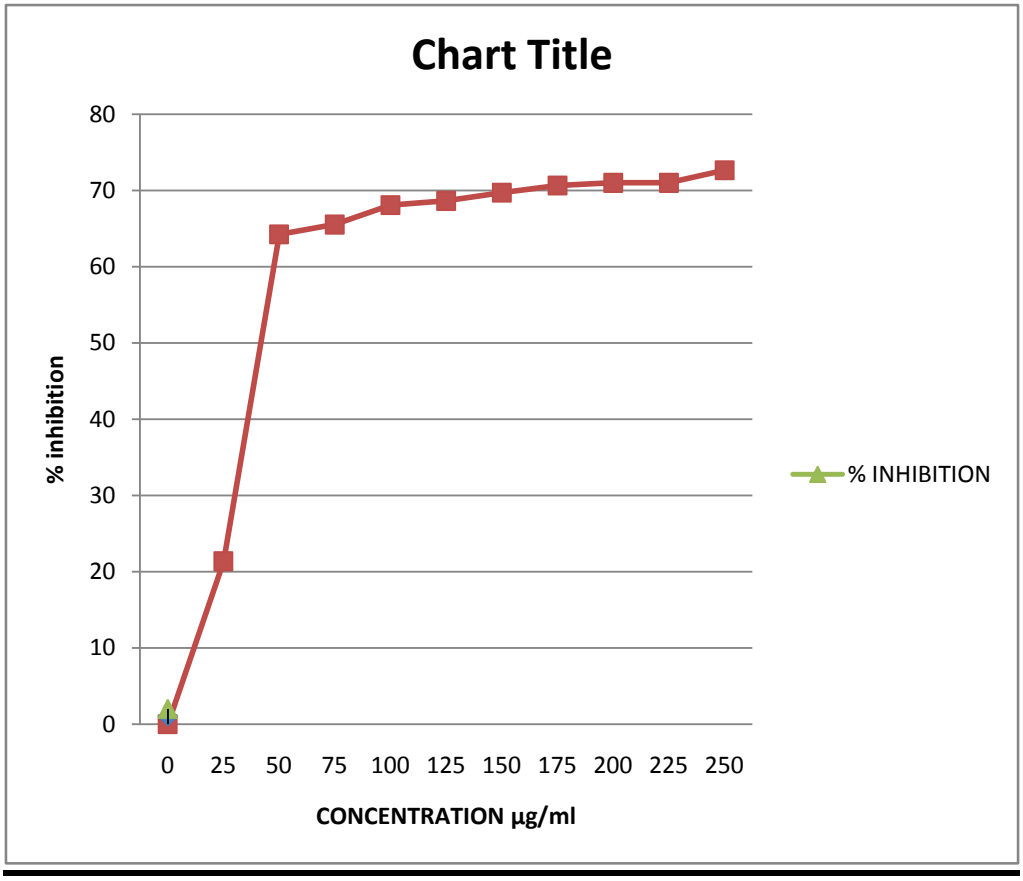


Fig VIII.3

Hydrogen peroxide method of Methyl salicylate (Standard)

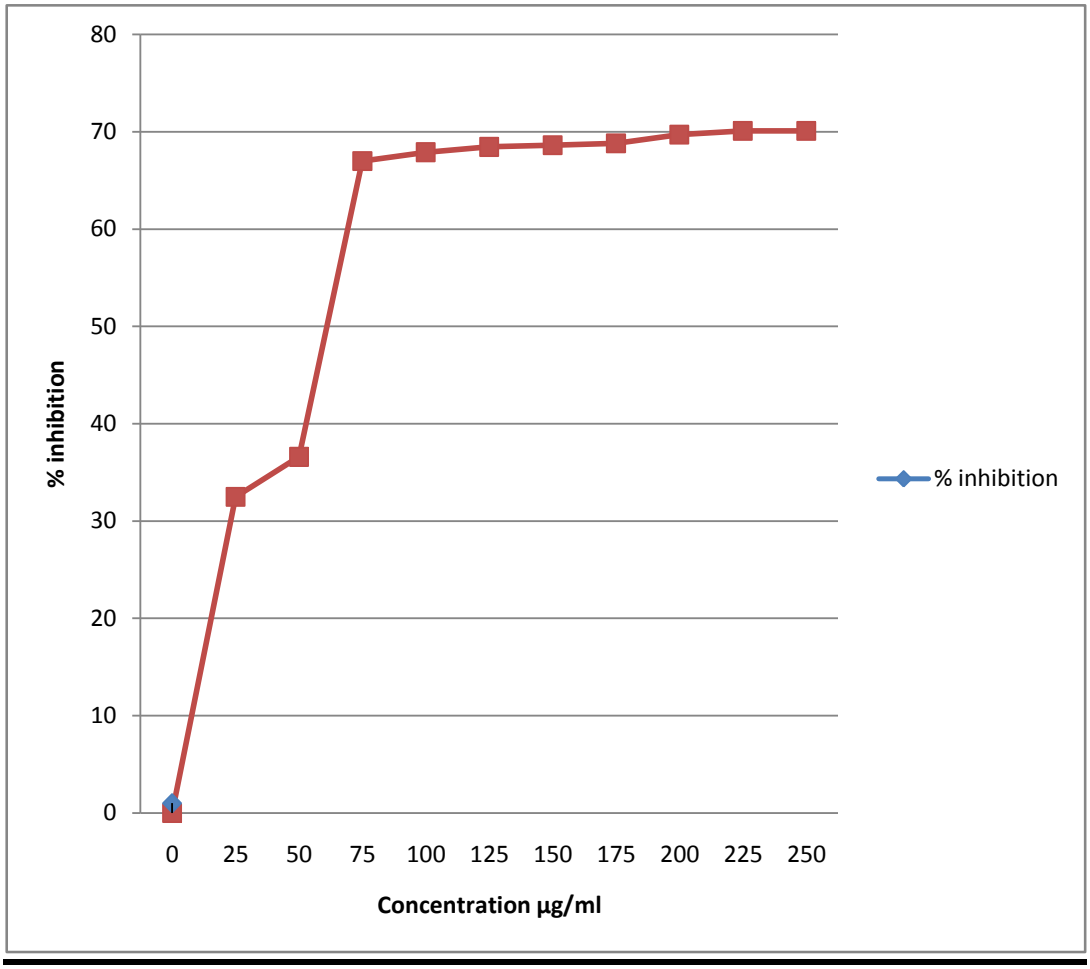


Fig VIII.4

Hydrogen peroxide method of Gallic acid(Standard)

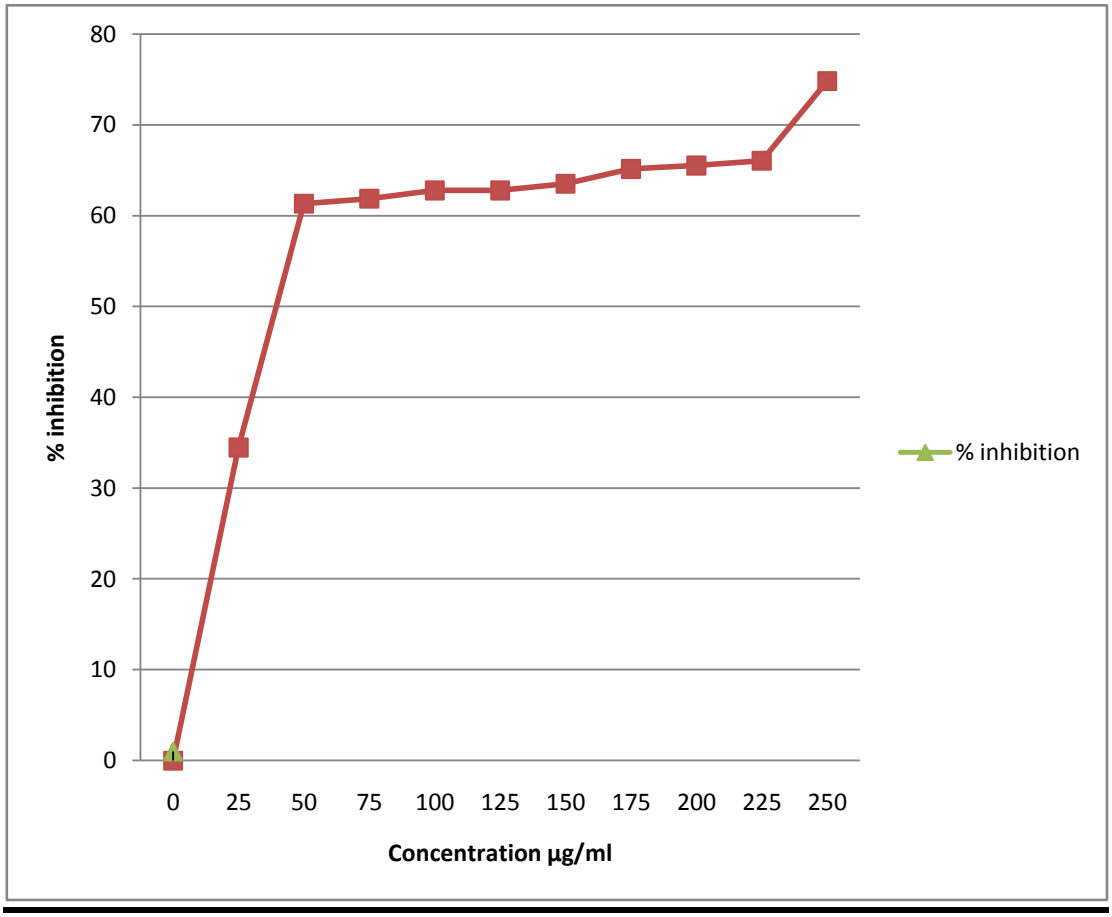


Fig VIII.5

COMPARISON GRAPH OF HYDROGEN PEROXIDE METHOD

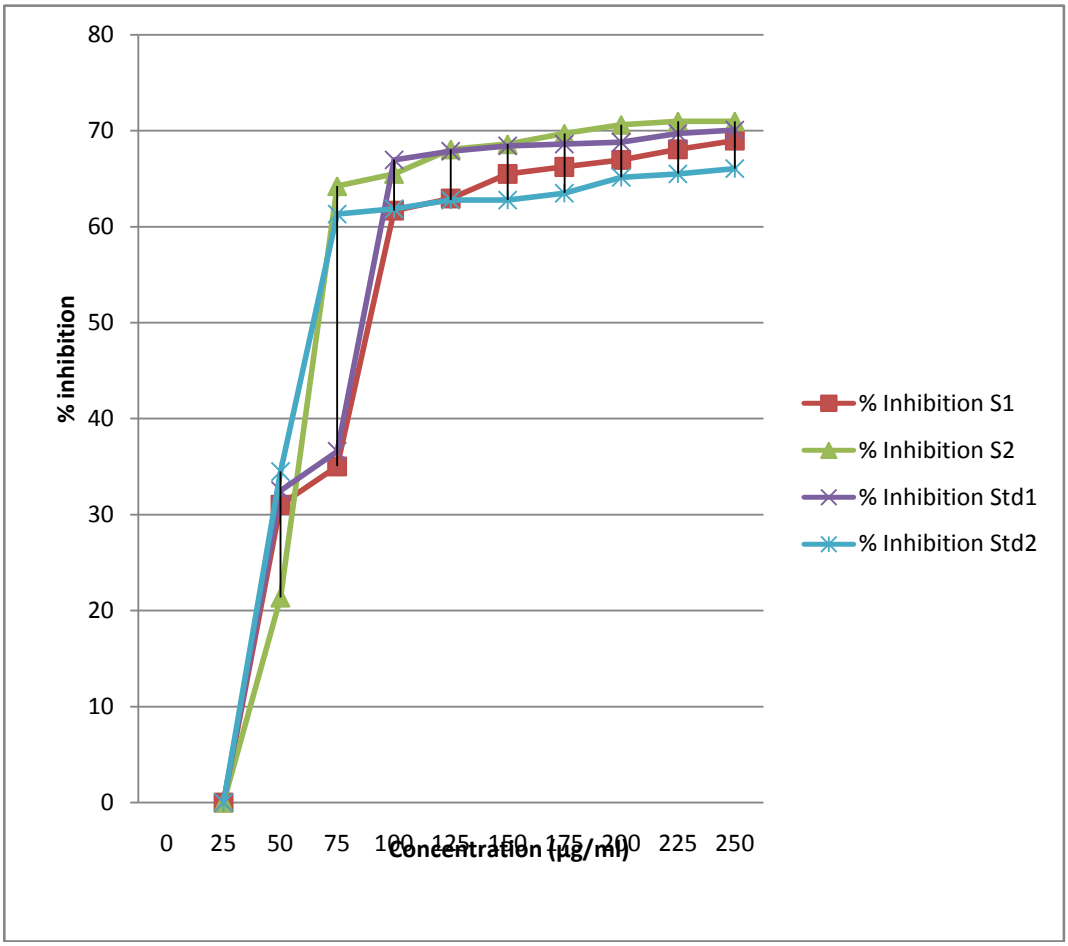


Fig VIII.6

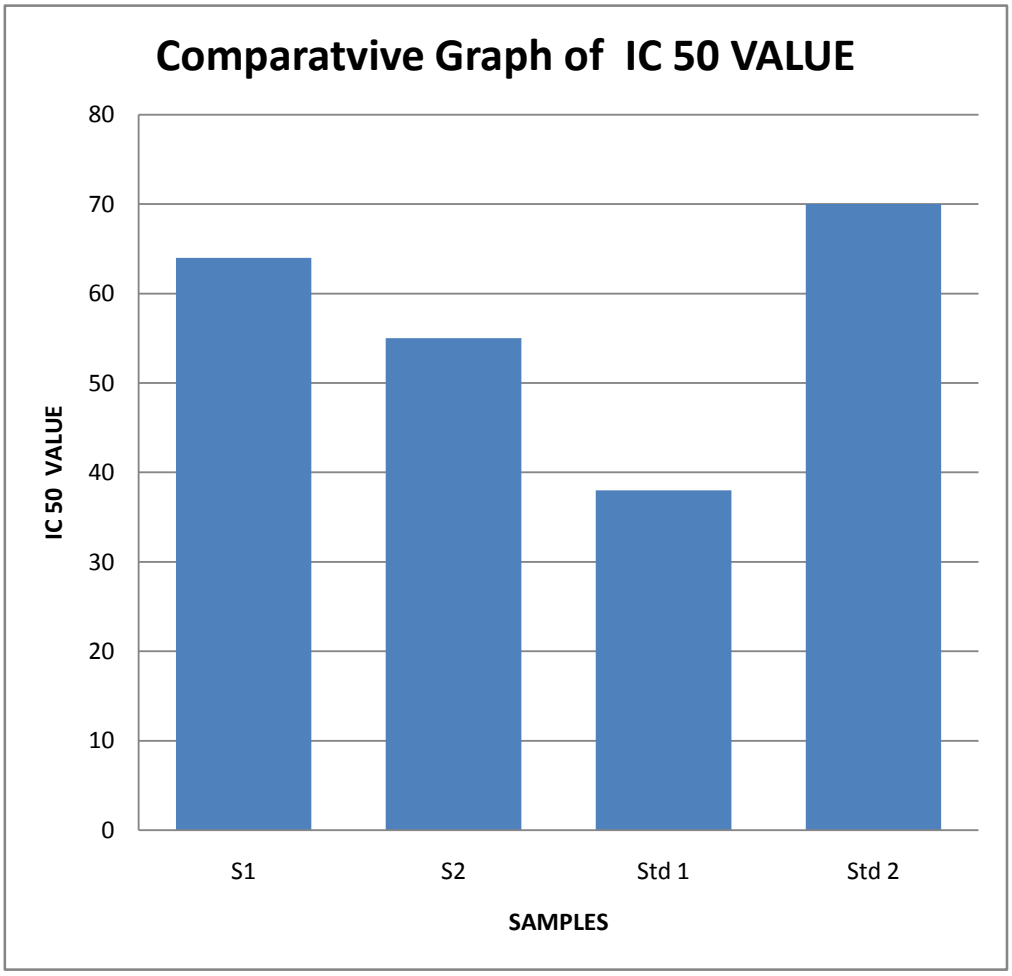

Comparatvive Graph of IC 50 VALUE
80
70
60
50
40
30
20
10
0
IC 50 VALUE
S1
S2
Std 1
Std 2
SAMPLES

Fig VIII.7

DPPH METHOD

The result of free radicals scavenging activity of methanolic extract of the two plants and the positive control gallic acid in DPPH free redical system were shown in table 1,2,&3.

Phyllanthus emblica

Table No 18

Sr. No.	Concentration (µg/ml)	Absorbance			Average	% Inhibition
		1	2	3		
1	50	0.522	0.520	0.521	0.521	18.30
2	100	0.410	0.405	0.407	0.407	43.78
3	150	0.135	0.137	0.134	0.135	81.35
4	200	0.084	0.082	0.082	0.082	88.67
5	250	0.047	0.047	0.046	0.047	93.50
6	500	0.037	0.036	0.036	0.036	95.02
7	1000	0.034	0.033	0.032	0.033	95.44
8	1500	0.032	0.033	0.032	0.032	95.58
9	2000	0.032	0.033	0.032	0.032	95.58
10	2500	0.032	0.031	0.032	0.032	95.58

Phyllanthus amarus

Table No 19

Sr. No.	Concentration (µg/ml)	Absorbance			Average	% Inhibition
		1	2	3		
1	50	0.582	0.583	0.582	0.582	19.61
2	100	0.510	0.512	0.510	0.510	29.55
3	150	0.420	0.421	0.422	0.421	41.85
4	200	0.330	0.331	0.333	0.331	54.28
5	250	0.284	0.283	0.284	0.284	60.77
6	500	0.108	0.109	0.111	0.109	84.94
7	1000	0.043	0.044	0.043	0.043	94.06
8	1500	0.043	0.045	0.044	0.044	93.93
9	2000	0.035	0.037	0.036	0.036	95.02
10	2500	0.030	0.032	0.034	0.032	95.10

GALLIC ACID (Standard)

Table No 20

Sr. No.	Concentration (µg/ml)	Absorbance			Average	% Inhibition
		1	2	3		
1	10	0.300	0.301	0.300	0.300	58.97
2	20	0.121	0.121	0.123	0.121	83.28
3	30	0.022	0.021	0.023	0.022	96.96
4	40	0.024	0.023	0.022	0.022	96.96
5	50	0.021	0.020	0.021	0.021	97.09

Table No. 21

Phyllanthus emblica	
Concentration µg/ml	**% inhibition**
0	0.00
50	18.30
100	43.78
150	81.35
200	88.67
250	93.50
500	95.02
1000	95.44
1500	95.58
2000	95.58
2500	95.58

TABLE No.22

Phyllanthus amarus	
Concentration µg/ml	% inhibition
0	0.00
50	28.61
100	29.55
150	41.85
200	54.28
250	60.77
500	84.94
1000	94.06
1500	93.92
2000	95.02
2500	95.10

Table No. 23

GALLIC ACID (STANDARD)	
Concentration µg/ml	**% inhibition**
0	0.00
10	58.97
20	83.28
30	96.96
40	96.96
50	97.09

The I.C 50 of various species were found to be as follows :

Table NO. 24

Samles	IC 50
Phyllanthus emblica	110µg/ml
Phyllanthus amarus	180µg/mi

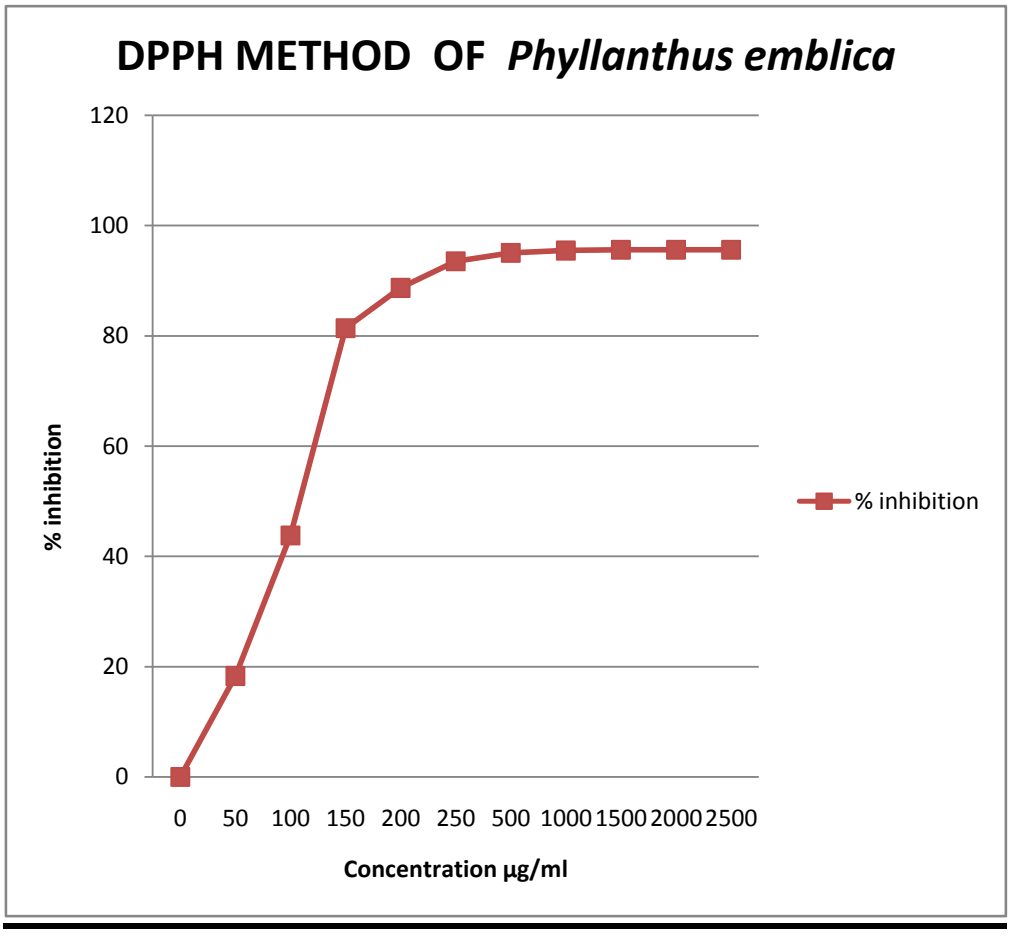

DPPH METHOD OF Phyllanthus emblica
120
100
80
60
40
20
0
% inhibition
0
50
100
150
200
250
500
1000
1500
2000
2500
Concentration µg/ml
% inhibition

Fig VIII.9

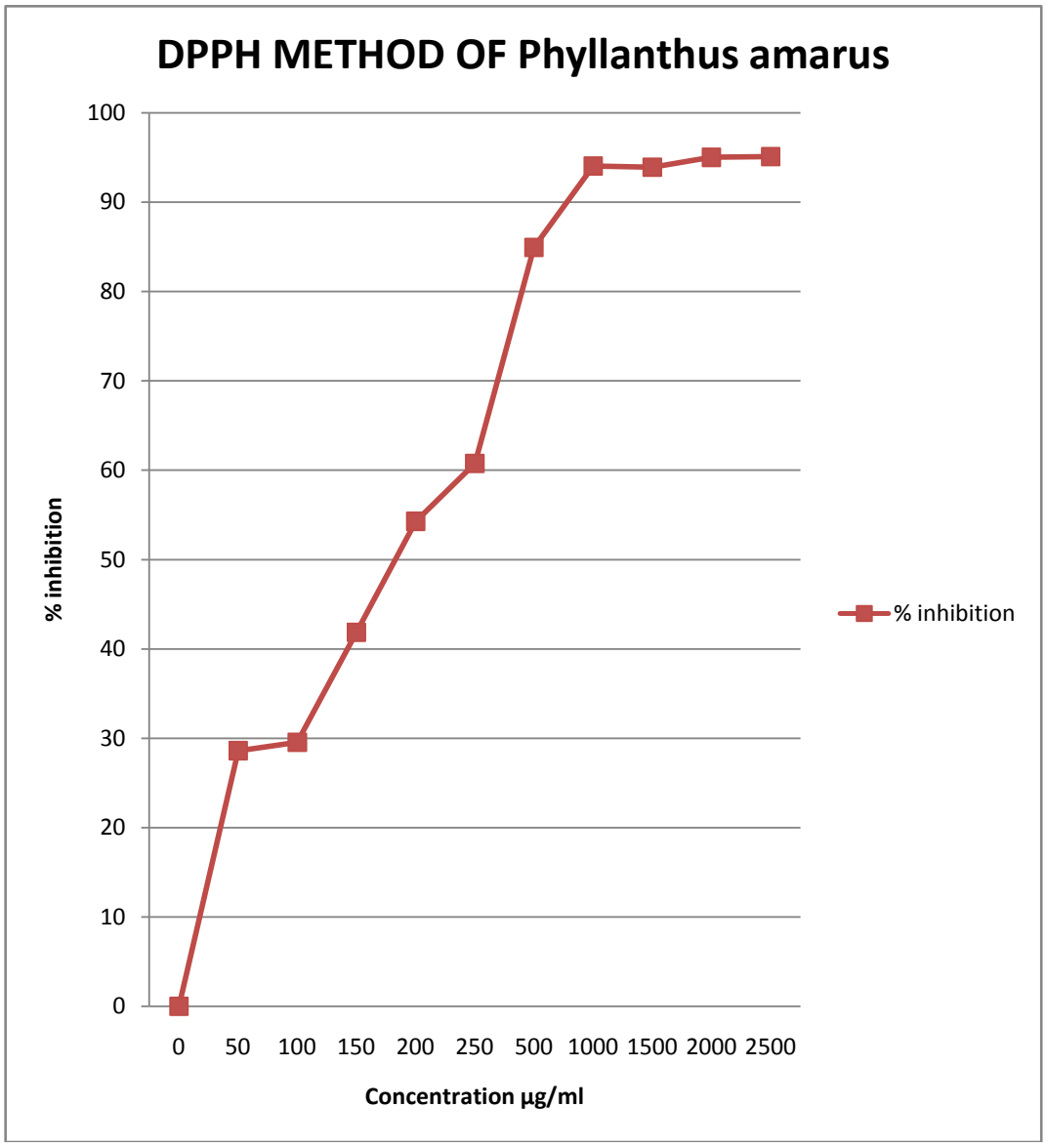

DPPH METHOD OF Phyllanthus amarus
100
90
80
70
60
50
40
30
20
10
0
% inhibition
0
50
100
150
200
250
500
1000
1500
2000
2500
Concentration µg/ml
% inhibition

Fig VIII.10

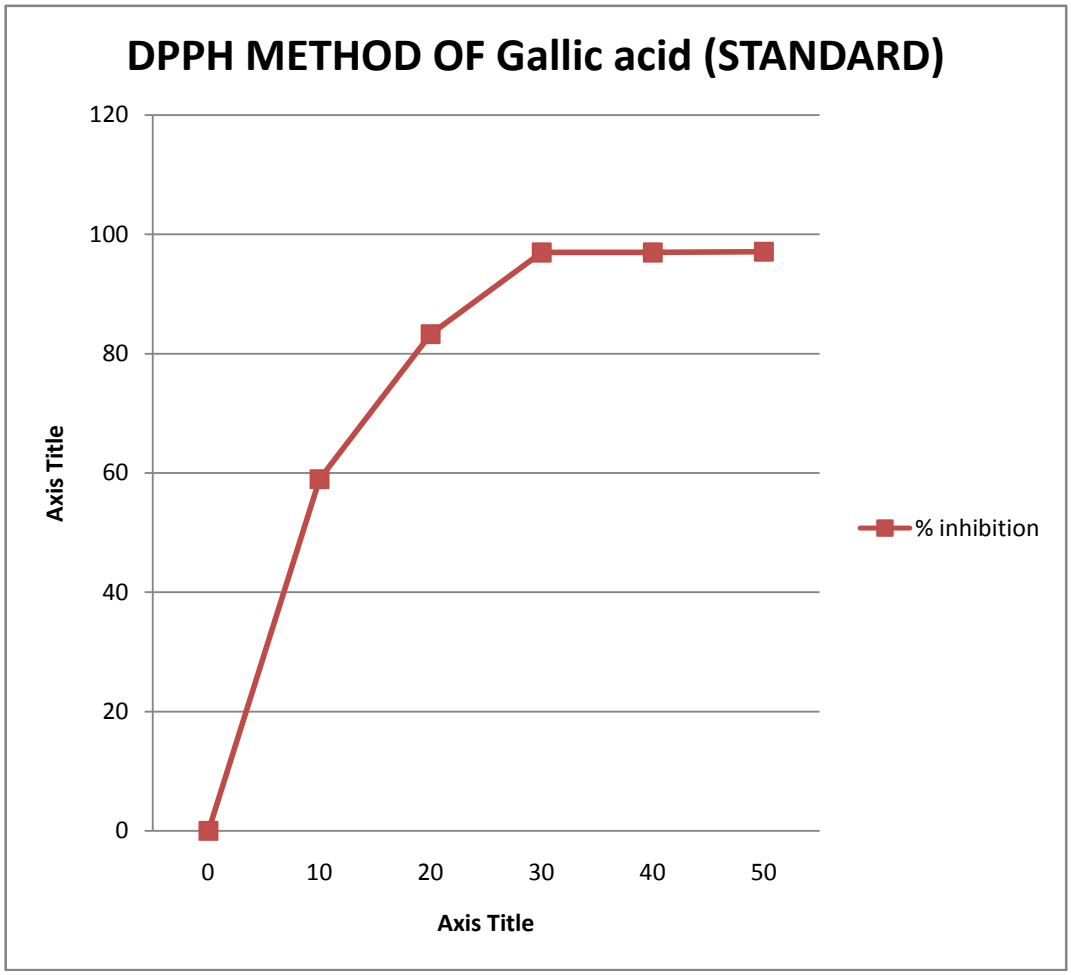

DPPH METHOD OF Gallic acid (STANDARD)
120
100
80
60
40
20
0
Axis Title
0
10
20
30
40
50
Axis Title
% inhibition

Fig VIII.11

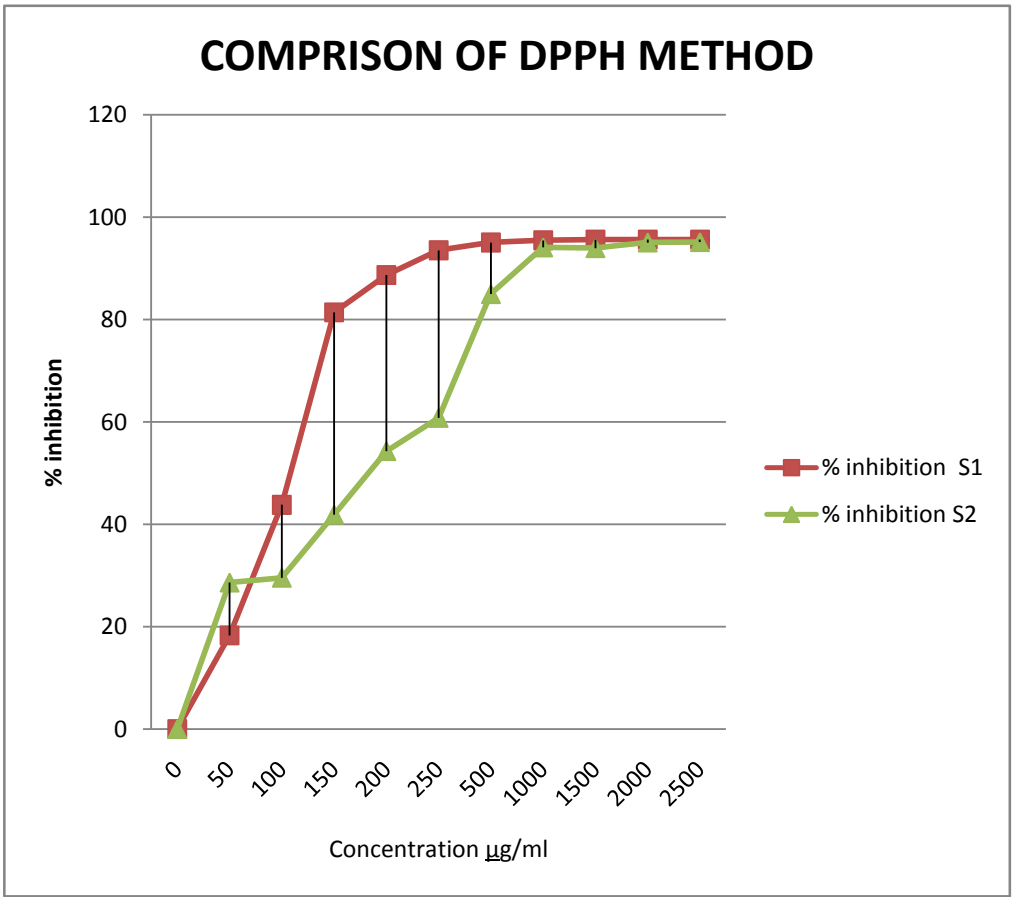

COMPRISON OF DPPH METHOD
120
100
80
60
40
20
0
% inhibition
0
50
100
150
200
250
500
1000
1500
2000
2500
Concentration µg/ml
% inhibition S1
% inhibition S2

Fig VIII.12

COMPARITIVE GRAPH FOR IC_{50} VALUE

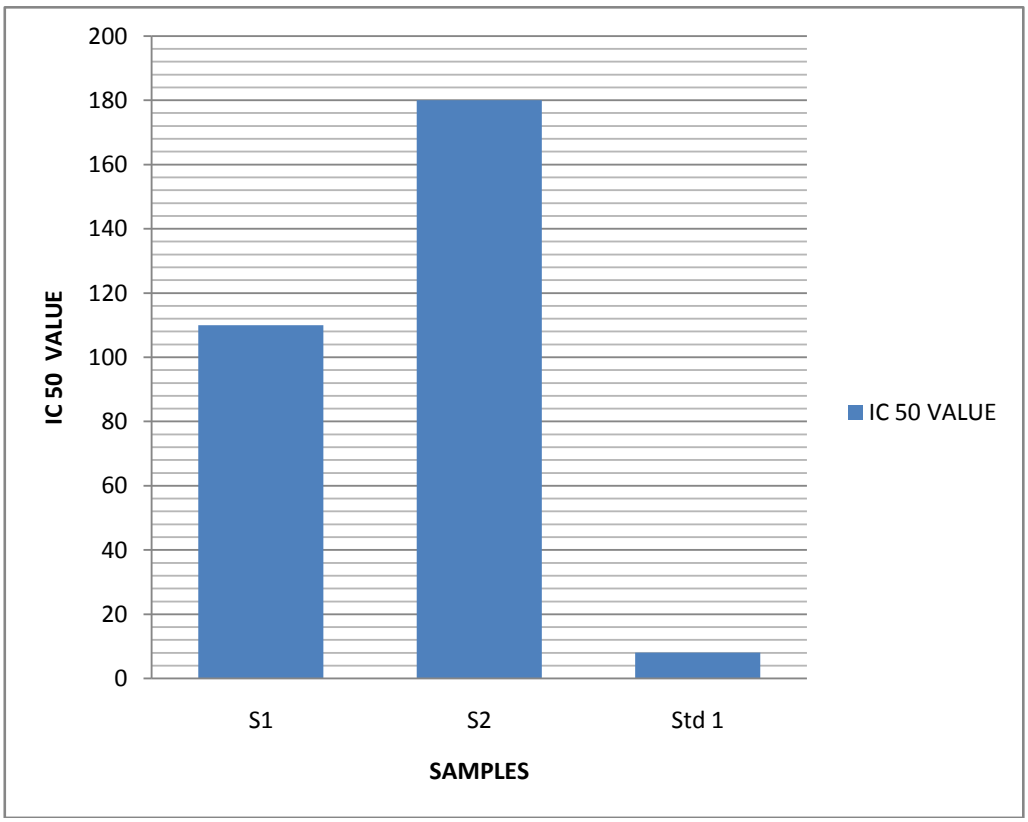


Fig VIII.13

Antiepileptic activity

Maximal electroshock induced convulsion

Effects of MEPE and MEPN on MES induced Epilepsy

Phenytoin treated animals have shown 100% protection against MES induced seizures where as MEPE 200mg/kg and MEPN 200 mg/kg have shown 54.35% and 67.31% protection respectively against MES induced seizures.

The MEPE 200mg/kg and MEPN 200 mg/kg at both doses and standard treated rats did not show any significant change in duration of tonic flexion and clonic convulsions. MEPE 200mg/kg and MEPN 200 mg/kg had shown a significant decrease in the duration of tonic extensor phase and comparable $p<0.001$ with the standard.

Results are shown in **Table 25** and **figure VII.14.**

Effect of MEPE and MEPN on neurotransmitter levels in MES induced rats

Serotonin

A significant $p<0.001$ & $p<0.001$ decrease in brain Serotonin levels was observed in forebrain of epileptic control animals. MEPE and MEPN 200mg/kg and 200 mg/kg treated rats have shown a significant $p<0.001$, $p<0.001$ increase in Serotonin levels in forebrain. The results are shown in **Table 26** and **figure VII.15**

Nor adrenaline

A significant $p<0.001$ decrease is observed in forebrain in epileptic control animals. MEPE 200mg/kg and MEPN 200 mg/kg and PHT treated animals showed a significant $p<0.05$ & $p<0.001$ increase in Nor adrenaline levels in forebrain of MEPE and MEPN 200mg/kg and 200 mg/kg treated animals. Results are shown in **Table 27** and **figure VII.16.**

Dopamine

A significant $p<0.001$ decrease in the dopamine levels is observed in forebrain in epileptic control animals and a significant $p<0.001$ increase is observed in forebrain on MEPE and MEPN 200mg/kg and 200 mg/kg treated rats, PHT treated animals showed a significant $p<0.001$ increase forebrain. Results are shown in **Table 28** and **figure VII.17.**

Effect of MEPE and MEPN on MES induced epilepsy

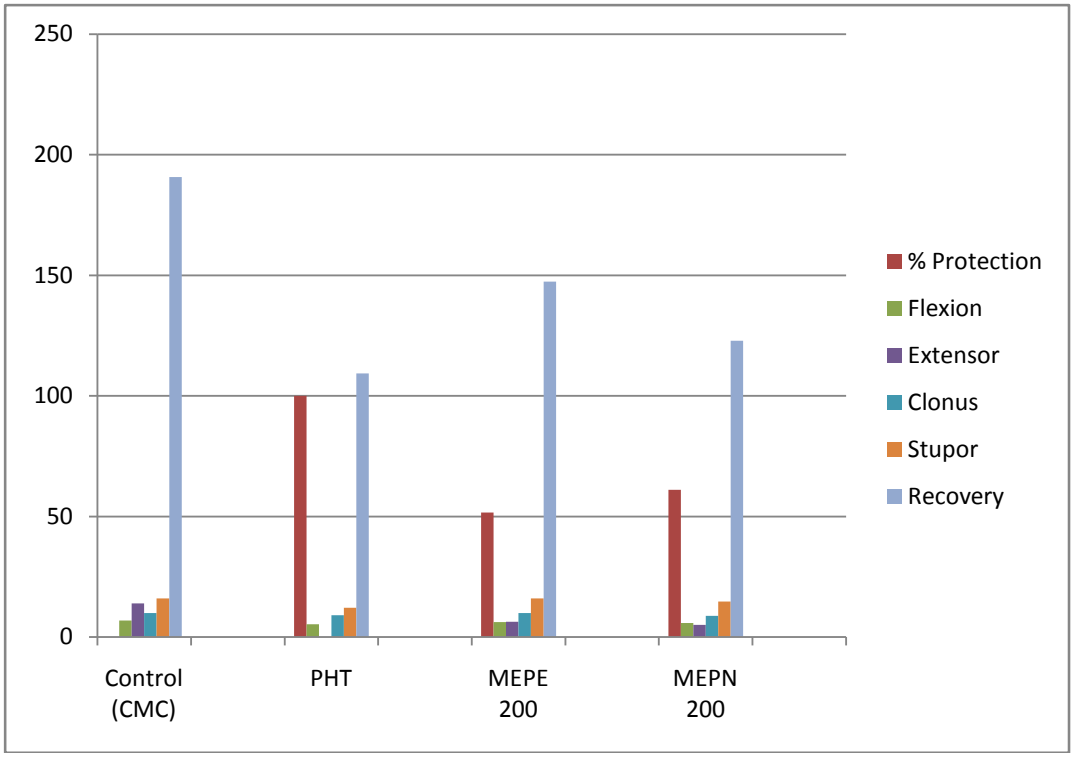
Table No. 25

Sr. No	Groups	% Protection	Flexion	Extensor	Clonus	Stupor	Recovery
I.	Control (CMC)	0	6.87±0.41	13.89±0.81	9.89±0.52	16±0.32	190.82
II.	PHT	100	5.27±0.38	0	9±0.32**	12.17±0.58***	109.34
III.	MEPE 200	51.69	6.18±0.35$_{ns}$	6.28±0.61***	10±0.43$_{ns}$	15.98±0.47$_{ns}$	147.35
IV.	MEPN 200	61	5.87±0.37***	5.07±0.37***	8.78±0.55$_{ns}$	14.68±0.57$_{ns}$	122.81

Values are expressed as mean± SEM of six observations. Comparison between Group I Vs Group II, Group II Vs Group II & Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's't' test *$p<0.05$; ** $p<0.01$; ***$p<0.001$; ns-non significant. Values expressed in seconds.

Fig VIII.14

Graph for Effect of MEPE and MEPN on MES induced epilepsy



Effect of MEPE and MEPN on Serotonin levels in MES induced epilepsy

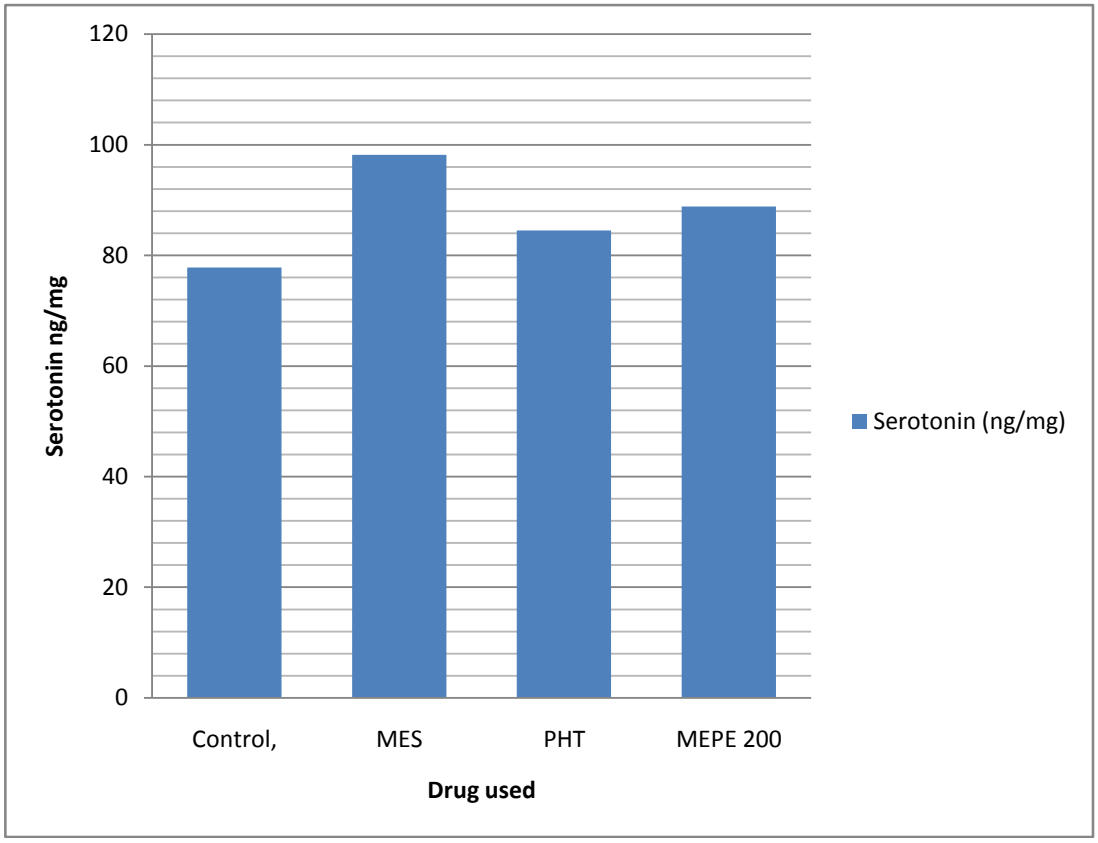
Table: 26

Groups	Drug used	Serotonin (ng/mg)
I	Control	l 181.33 ± 1.84
II	MES	77.83 ± 1.34a***
III	PHT	98.16 ± 0.9b**⁎
IV	MEPE 200	84.5 ± 1.73b***
V	MEPN 200	88.83 ± 1.02b***

Values are expressed as mean ± SEM of six observations. Comparison between Group I Vs Group II, Group III Vs Group IV & Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test *p<0.05; ** p<0.01; ***p<0.001; ns-non significant. Units = ng/mg of wet tissue

Fig VIII.15

Graph for Effect of MEPE and MEPN on Serotonin levels in MES induced epilepsy



Effect of MEPE and MEPN on Non adrenaline levels in MES induced epilepsy

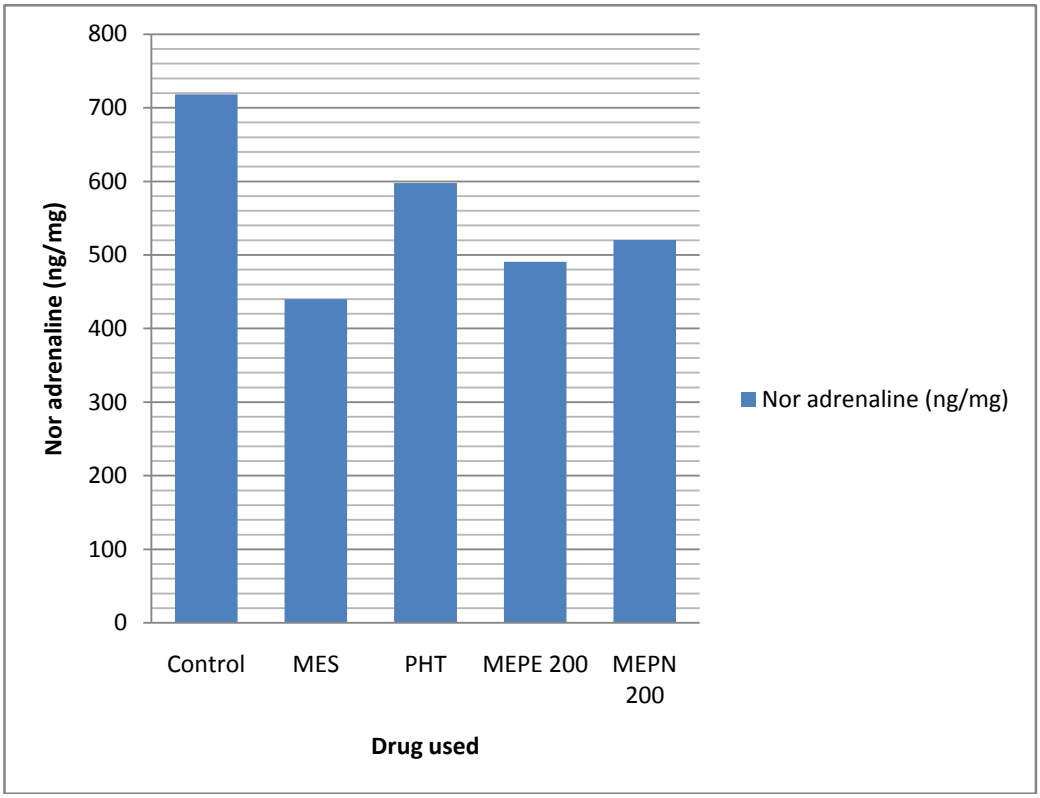
Table No. 27

Groups	Drug used	Nor adrenaline (ng/mg)
I	Control	718.3 ± 2.50
II	MES	440.16 ± 1.02a***
III	PHT	597.66 ± 1.90b***
IV	MEPE 200	490.83 ± 3.17b***
V	MEPN 200	520.3 ± 2.52b***

Values are expressed as mean± SEM of six observations. Comparison between Group I Vs Group II, Group III Vs Group IV & Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. *p<0.05; ** p<0.01; ***p<0.001; ns-non significant. Units = ng/mg of wet tissue.

Fig VIII.16

Graph for Effect of MEPE and MEPN on Non adrenaline levels in MES induced epilepsy



Effect of MEPE and MEPN on Dopamine levels in MES induced epilepsy

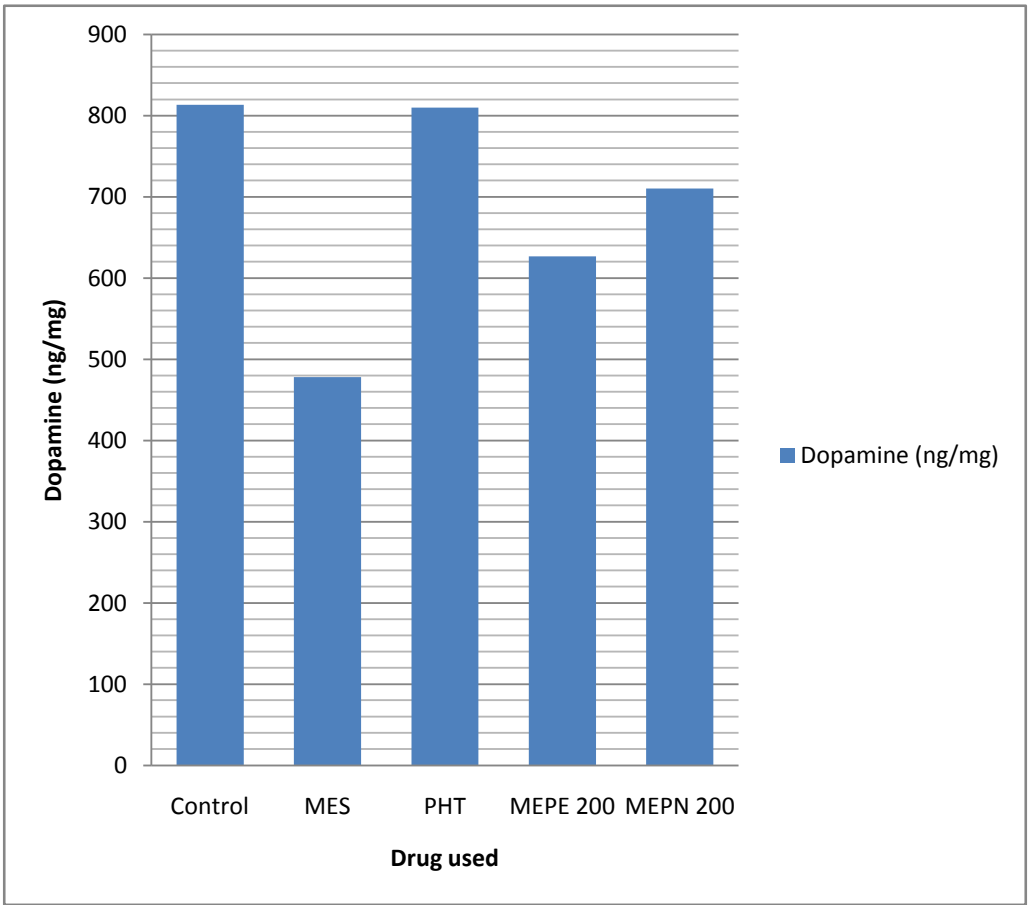
Table NO. 28

Groups	Drug used	Dopamine (ng/mg)
I	Control	813.3 ± 0.84
II	MES	478.16 ± 1.29a***
III	PHT	810 ± 1.12b***
IV	MEPE 200	626.66 ± 3.45b***
V	MEPN 200	710.3 ± 2.71b***

Values are expressed as mean± SEM of six observations. Comparison between Group I Vs Group II, Group III Vs Group IV & Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. *p<0.05; ** p<0.01; ***p<0.001; ns-non significant. Units = ng/mg of wet

Fig VIII.17

Graph for Effect of MEPE and MEPN on Dopamine levels in MES induced epilepsy



DISCUSSION

A) EXTRACTIVE VALUE

- The Petroleum ether soluble extractive value of samples was found to be

  S1 = 0.3%w/w

  S2 = 2.8

- The Benzene soluble extractive value of samples was found to be

  S1 = 13.1%w/w

  S2 = 14.9%w/w

- The Chloroform ether soluble extractive value of samples was found to be

  S1 = 1.3%w/w

  S2 = 0.8%w/w

- The Methanol ether soluble extractive value of samples was found to be

  S1 = 24%w/w

  S2 = 16%w/w

- The Water soluble extractive value of samples was found to be

  S1 = 4.6%w/w

  S2 = 5.2%w/w

B) ASH VALUE

- The Total ash value of the samples was found to be

  S1 = 4.6w/w

  S2 = 5.4w/w

- The Acid insoluble ash value of the samples was found to be

  S1 = 2.8w/w

  S2 = 1.3w/w

- The Water insoluble ash value of the samples was found to be

  S1 = 1.2w/w

  S2 = 1.5w/w

- The Sulphated ash value of the samples was found to be

S1 = 3.5w/w

S2 = 2.4w/w

C) FIBRE CONTENT

- The fibre content of the samples was found to be

S1 = 0.103%w/w

S2 = 0.416%w/w

D) PHYTO-CHEMICAL TEST

The quantitative phytochemical analysis of samples was showed the presence of majority of the compounds like

- Alkaloids
- Carbohydrates
- Proteins and Free amino acids
- Tannins and Phenolic Compounds
- Steroidal glycosides
- Flavonoids

E) HPTLC FINGER PRINTS

- The R_F value of the methanolic extract of ***Phyllanthus emblica*** was found to be : **1.32**
- The R_F value of the methanolic extract of ***Phyllanthus amarus*** was found to be : **1.29**
- The R_F value of the methanolic extract of Gallic acid was found to be : **1.37**
- The R_F value of the methanolic extract of Methyl salicylate was found to be : **0.12**

F) ESTIMATION OF VITAMIN C

- The amount of vitamin c was found to be

S1 = 0.68gm/25ml

S2 = 52gm/25ml

G) ESTIMATION OF ANTI OXIDANT ACTIVITY

1) HYDROJEN PEROXIDE METHOD

- The IC_{50} value of the sample ***Phyllanthus emblica*** was found to be : 64 µg/mi
- The IC_{50} value of the sample ***Phyllanthus amarus*** was found to be : 55µg/mi

2) DPPH METHOD

- The IC_{50} value of the sample ***Phyllanthus emblica*** was found to be : 110 µg/mi
- The IC_{50} value of the sample ***Phyllanthus amarus*** was found to be : 180 µg/mi

ANTIEPILEPTIC ACTIVITY

1) Effects of MEPE and MEPN on MES induced Epilepsy

- Phenytoin treated animals have shown 100% protection against MES induced seizures where as MEPE 200mg/kg and MEPN 200 mg/kg have shown 54.35% and 67.31% protection respectively against MES induced seizures

2) Effect of MEPE and MEPN on neurotransmitter levels in MES induced rats

A) **Serotonin**

- A significant $p<0.001$ & $p<0.001$ decrease in brain Serotonin levels was observed in forebrain of epileptic control animals. MEPE and MEPN 200mg/kg and 200 mg/kg treated rats have shown a significant $p<0.001$, $p<0.001$ increase in Serotonin levels in forebrain

B) **Nor adrenaline**

- A significant $p<0.001$ decrease is observed in forebrain in epileptic control animals. MEPE 200mg/kg and MEPN 200 mg/kg and PHT treated animals showed a significant $p<0.05$ & $p<0.001$ increase in Nor adrenaline levels in forebrain of MEPE and MEPN 200mg/kg and 200 mg/kg treated animals.

C) **Dopamine**

- A significant $p<0.001$ decrease in the dopamine levels is observed in forebrain in epileptic control animals and a significant $p<0.001$ increase is observed in forebrain on MEPE and MEPN 200mg/kg and 200 mg/kg treated rats, PHT treated animals showed a significant $p<0.001$ increase forebrain.

CHAPTER - IX

Conclusion

CONCLUSION

In the present study, two methods were used for evaluation of antioxidant activity. The alcoholic extract of three plants from the Euphorbiaceae family showed good antioxidant activity where the samples 1 & 2 showed very good activity compared with the standard. The antioxidant properties of the two species are in the following order.

In hydrogen peroxide method ***Phyllanthus amarus extract*** showed potent antioxidant activity comapred to ***Phyllathus emblica.***

Phyllathus emblica < Phyllanthus amarus

In DPPH method ***Phyllathus emblica*** showed potent antioxidant activity compared to ***Phyllanthus amarus.***

Further studies are warranted for the isolation and identification of individual phenolic compound and also in-vivo studies are needed for understanding their mechanism of action as an anti-oxidant better.

In epilepsy, normal pattern of neuronal activity becomes disturbed briefly when the nerves in the brain "Fire" spontaneously causing strange sensations, emotions, behaviours and often times seizures with muscle spasms as well as loss of consciousness[46]

It has been reported to increase the brain levels of Dopamine and Nor adrenaline which causes an inhibition of seizure activity[47]

MES induced epilepsy was altering the levels of monoamines like nor adrenaline, serotonin, dopamine[48]**.**

It is found that treatment with Methanolic Extract of ***Phyllanthus emblica*** and Methanolic Extract of ***Phyllanthus amarus*** on rats significantly reduces in tonic hind limb extensor stage in **Maximal electroshock** induced epilepsy. Methanolic Extract of both the plants markedly protects epilepsy induced by MES which are mediated by levels of monoamines.

CHAPTER - X

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